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THE PULMONARY FUNGUS HAPLOSPORANGIUM PARVUM. II. STRAIN AND GENERIC RELATIONSHIPS [1]

J. W. CARMICHAEL

(WITH 4 FIGURES)

In Arizona in 1942, Emmons and Ashburn (**12**) discovered in the lungs of mice and ground squirrels spherical bodies about 14 microns in diameter. These bodies resembled the sporangia of *Coccidioides immitis,* with which they were frequently associated. When cultured on nutrient medium, however, they gave rise to a fungus which the authors described as a new species, *Haplosporangium parvum.* Colonies of *H. parvum* were white or cream-colored with a cottony layer of aerial hyphae. The mold was characterized as having delicate, frequently-branching hyphae and stipitate, subspherical, hyaline conidia. The conidia were borne singly, or rarely in chains of two, at the ends of hyphae, or at the tips of short lateral conidiophores.

In Alberta in 1946, Dowding (**8**) discovered, in the lungs of mice and a squirrel, large, thick-walled, spherical bodies attaining a diameter of 300 microns. To her surprise, these bodies when cultured produced a mold indistinguishable from the *Haplosporangium* of Emmons and Ashburn. The bodies in the lungs she called chlamydospores.

[1] A continuation of research on *Haplosporangium* being carried out at the Provincial Laboratory of Public Health, Edmonton, Canada, under the direction of E. Silver Dowding. This investigation was made possible by financial assistance from the Department of Health of the Dominion of Canada.

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Since then, Jellison has found these large cells of *Haplosporangium* in many Montana animals, including rock rabbits, weasels, and skunks, as well as beaver and various other rodents. His recent paper (**15**) describes these discoveries and reviews reports of *Haplosporangium* from Minnesota, Texas, California, and British Columbia.

There is a tremendous difference between the size of the chlamydospores found in the lungs of Arizona and Texas rodents, and those found in the lungs of Montana and Alberta animals. Chlamydospores of southern strains are 10 to 14 microns in diameter while chlamydospores of northern strains range from 110 to 390 microns. Because of this difference in size it has been suggested by Emmons (personal communication) and Jellison (**15**) that the *Haplosporangium* occurring in Montana and Alberta may be a different species than the one originally described from Arizona.

To determine more clearly the relationship between northern and southern strains, fifteen cultures of *Haplosporangium* were obtained from Arizona, Texas, Montana, Idaho and Alberta animals, and their characters compared on culture media and in laboratory animals. Study of the fifteen strains also disclosed new morphological aspects which may help to clarify the generic relationships of *H. parvum*.

MATERIALS AND METHODS

Strains 1 to 3 came from animals with small chlamydospores in the lungs, while strains 4 to 15 came from animals with large chlamydospores (with the possible exception of Montana strain 14 which was not seen in the host (**15**) and was atypical in experimental animals). The seven Alberta strains came from prairie, parkland, and forest regions of the Province. Three of the four Montana strains were collected not from rodents, but from carnivores. Strains 1–3 were kindly sent by Dr. C. W. Emmons, strains 11–15 by Dr. W. L. Jellison. Details of the sources of the strains are shown in TABLE I.

Cultures were grown on Sabouraud's agar (Difco), Littman's agar (Difco) (**17, 18**), corn-meal agar (**4**), and blood agar. Room temperature varied from less than 10° C. at night to more than 30° C. on hot days. Cultures at elevated temperatures were made

TABLE 1

FIFTEEN STRAINS OF HAPLOSPORANGIUM PARVUM

Strain	Location	Host	Collector and Number	
1	Arizona	?	Emmons	?
2	Arizona	?	Emmons	2370
3	Texas	pack rat	Emmons	2372
4	Alberta	deer mouse	Dowding	184
5	Alberta	deer mouse	Dowding	19
6	Alberta	deer mouse	Dowding	10
7	Alberta	deer mouse	Dowding	17
8	Alberta	deer mouse	Dowding	190
9	Alberta	deer mouse	Dowding	F29
10	Alberta	deer mouse	Dowding	50–59
11	Montana	weasel	Jellison	27258
12	Montana	muskrat	Jellison	27242
13	Montana	skunk	Jellison	27128
14	Montana	weasel	Jellison	27274
15	Idaho	deer mouse	Jellison	27097A

by transferring fragments from 2–8-week-old Sabouraud-agar-slant cultures to blood agar plates. These were incubated in the inverted position, and water was added to the lids as necessary to prevent drying.

Microscopic examination was made on material mounted in 85% lactic acid 2 parts, and Lugol's iodine 1 part.

The spores often varied considerably in size in a single mount, especially chlamydospores. Therefore, to avoid personal influence in selection, all spores in focus at one time were measured. The spores were considered to be isodiametric and were measured without reference to their point of attachment to the conidiophore.

Nuclei were stained by DeLamater's method (**7**).

Animal injections were made with spore suspensions which were prepared by adding about 10 cc. of sterile, normal saline to Sabouraud-agar-slant cultures (2–8 weeks old) and scraping the surface growth with a loop. The bottles were then shaken gently and the suspensions checked microscopically to be sure spores were present. Animals were injected subcutaneously on each side of the abdomen using 0.2 cc. of suspension for each injection.

Since the *Haplosporangium* spores remained localized after injection, and developed in the subcutaneous tissues, their development could be followed without resorting to embedding and sectioning. Small pieces of subcutaneous tissue from the injection

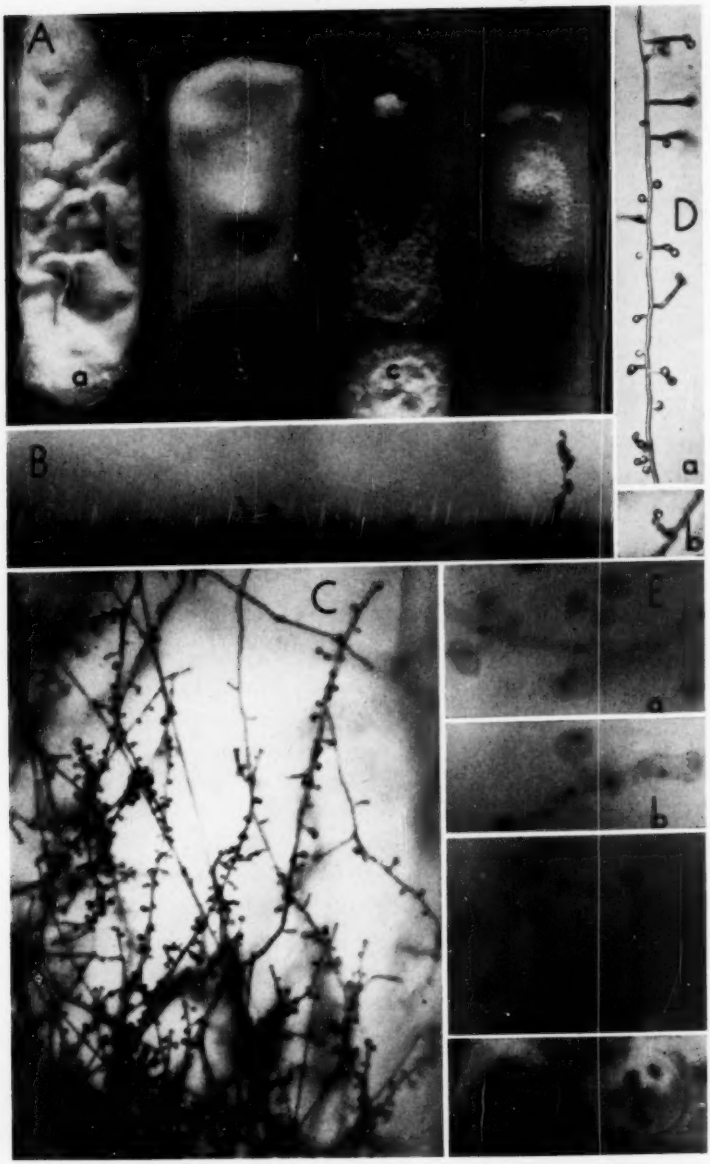

FIG. 1.

sites were teased apart and examined in 10% potassium hydroxide or lacto-phenol cotton-blue, or, for microcultures, on Sabouraud's agar. Tissue sections were also cut and stained in the usual manner. The writer is indebted to Dr. D. O. W. Waugh of the Department of Pathology for examining the slides.

PART 1: STRAIN RELATIONSHIPS

CULTURES COMPARED AT ROOM TEMPERATURE

Colony Types. On the basis of colonial morphology on Sabouraud's agar, cultures of the fifteen strains of *Haplosporangium* can be classified into four types (FIG. 1A), as follows:

Granular type colonies have a short nap of aerial mycelium (1 mm. deep or less) which has a plaster-like appearance reminiscent of *Trichophyton mentagrophytes*. The surface of the colony is heaped and thrown into folds. Spore production in these colonies is moderately high. Strains 1, 4, 5, 6, 8, 9, 10 and 15 have produced granular colonies.

Cottony type colonies have a dense, usually white felt of aerial mycelium. The texture and appearance of these colonies is very close to *Histoplasma capsulatum*. Spore production is low or absent. This colony type has been seen in Arizona strains 1, 2, 3 and Alberta strain 7.

Floccose type colonies have a loose, buff-colored aerial mycelium covering the surface of the colony. The aerial hyphae adhere in tufts, giving the appearance of sheep's wool. These colonies have high spore production and there are many secondary spores. Floccose colonies were seen mainly in Montana and Idaho strains, but also in Alberta strains 6 and 8.

Glabrous type colonies often have a central tuft of spines, but no other aerial mycelium. They are moist and circular, flat or

FIG. 1. *H. parvum* in culture (except D, which is *Blastomyces dermatitidis*). F. At 40° C., rest at room temperature. A. Types of cultures after three weeks: *a*, granular; *b*, cottony; *c*, floccose (with glabrous central zone); *d*, glabrous (some aerial growth). B. Erect fertile hyphae after 8 days. C, D. Microcultures showing fertile hyphae with chains of conidia. E. *a* to *c*, nuclei, in hyphae and conidia. F. Nuclei in chlamydospores of Arizona strain. Magnification: B and C. × 150; D. × 335; E, F. × 1500.

smoothly raised towards the center. The firm hyphal mat is almost the color of the agar.

This type of growth is usual in all young cultures, but often persists, especially when the agar is moist and the temperature is high. Cultures of all strains have been observed to remain in this condition for at least two months when transferred in hot weather. Old cultures of other types sometimes revert to the glabrous condition by collapse of the aerial mycelium. Many cultures maintain glabrous zones or sectors.

These four colony types are intergrading. Also one strain may show more than one type of colony when transferred under different conditions. Strain 1, for example, has produced glabrous, granular and cottony types of colonies. In general, however, granular colonies are typical of Alberta strains, cottony colonies of Arizona strains, and floccose colonies of Montana strains, while glabrous colonies are produced by all strains under some conditions.

Growth Rate. The growth rate of young colonies of *H. parvum* was measured on Sabouraud's agar at room temperature. After five days the minimum, average and maximum diameters of the colonies in millimeters were respectively: for three southern strains, 12, 12 and 12; five Montana strains, 3, 9 and 11; seven Alberta strains, 2, 6 and 9. After ten days they were: three Arizona strains, 24, 26 and 28; five Montana strains, 16, 18 and 22; seven Alberta strains, 9, 16 and 20. That is, the three southern strains grew considerably faster than the northern strains: Montana strains on the average grew somewhat faster than Alberta strains, but individual colonies showed exceptions.

Number and Size of Spores. When fifteen strains of *Haplosporangium parvum* were maintained in the laboratory on Sabouraud's-agar slants and transferred every three months, most of them consistently produced large numbers of spores. Four strains (the three southern strains and Alberta strain 7) became sterile. Transferring to fresh medium usually had no effect. Sometimes, however, a single transfer in a series of sterile ones would inexplicably produce many spores. Two southern sterile strains (1 and 2) were transferred to mouse and rabbit dung in a moist chamber, where both strains sporulated abundantly.

TABLE II

AVERAGE SPORE DIAMETERS OF EIGHT HAPLOSPORANGIUM STRAINS

Geographic group	Strain number	Average diameter of 20 spores in microns	Diameters, by geographic groups		
			Minimum	Average	Maximum
Alberta	8	4.2			
Alberta	4	3.6	2.5	3.8	5.5
Alberta	10	3.6			
Montana	14	4.1			
Montana	13	3.5	2.5	3.6	5.0
Montana	12	3.2			
Arizona	1	3.2	2.5	3.2	4.0
Arizona	2	3.1			

From three-month-old Sabouraud-agar-slant cultures at room temperature.

Eight of the fifteen strains of *Haplosporangium* were selected for a study of their spore size. TABLE II shows that when the average spore sizes are compared by geographic groups, Alberta strains average very slightly larger than Montana strains, and both are somewhat larger than Arizona strains. When the average spore sizes of the individual strains are examined, however, it can be seen that it would be impossible to "place" with assurance an unknown strain with a spore diameter of, say, 3.3 μ or 3.5 μ.

CULTURES COMPARED AT HIGHER TEMPERATURE

When the conidia of Alberta strains of *H. parvum* are maintained at 37° C. they enlarge to form chlamydospores similar to those found in the lungs of infected animals (**8**). To determine if other North American strains behaved similarly, fragments from cultures of four Montana strains, three southern strains, and an Alberta control strain were incubated at 37° C. The diameter of 20 spores of each strain was measured at intervals.

TABLE III shows that three of the Montana strains and the Alberta strain formed chlamydospores, while conidia of the fourth Montana strain (14) did not enlarge appreciably.

The three southern strains (1, 2, and 3) did not form chlamydospores. Their conidia swelled slightly and germinated within two

TABLE III

SPORE ENLARGEMENT (*in vitro*)

Source and strain number	Average spore diameter in microns				
	at room temperature	at 37° C		at 40° C	
		5 days	10 days	6 days	12 days
Alberta 10	3.6	7.6	18.1	8.5	9.7
Alberta 8	4.2	—	—	5.1	11.9
Montana 13	3.5	7.0	26.0	6.0	9.0
Montana 11	—	8.3	8.6	—	—
Montana 12	3.2	7.4	15.2	—	—
Montana 14	4.1	4.5	4.7	6.2	6.6
Arizona 1	3.2	germinates		—	—
Arizona 2	3.1	germinates		9.3	10.3
Arizona 3	—	germinates		11.3	12.1

days to give rise to the mycelial phase (FIG. 3F). Colonies formed at this temperature were atypical, being compact, moist and tan-colored. The hyphae became closely septate and conidia were not formed.

Since southern strains seemed more resistant to heat, they were incubated at a higher temperature, 40° C. At this temperature southern strains, too, formed chlamydospores. TABLE III shows the extent of enlargement at 40° C. of two southern strains as compared with two Alberta and two Montana strains. All strains except Montana 14 enlarged to about the same extent.

Representative strains from each geographic area (2, 5, 13 and 15) were incubated at 45° C. No enlargement or growth was observed after seven days. The conidia remained viable however and germinated when returned to room temperature. One strain tested (8) was killed in 24 hours at 50° C.

The following generalizations from these experiments seem justified. At 37° Alberta and Montana strains form chlamydospores whose size varies greatly with the strain. Arizona strains, which grow in the mycelial phase at 37°, form similar chlamydospores at a slightly higher temperature. Northern strains of *H. parvum* can be distinguished from southern strains by their lower optimum temperature for chlamydospore formation.

EXPERIMENTAL INFECTIONS COMPARED

To compare their development in animals, two Arizona strains, three Montana strains and five Alberta strains of *H. parvum* were injected subcutaneously into guinea pigs and white mice.

Northern Strains. All of the animals inoculated with Alberta and Montana strains (23 guinea pigs and 6 mice) developed subcutaneous nodules. These appeared within 2–10 days after inoculation and sometimes persisted as long as seven months (the longest time any animal was observed). They were apparent as firm swellings at the sites of injection. Excised nodules ranged up to two centimeters in diameter.

The nodules were situated in the dermis or the underlying muscle. Typically they consisted of an abscess, or a small granuloma, surrounded by an area of subacute and chronic inflammatory reaction and fibrosis. Foreign-body giant cells were usually numerous. Occasionally epithelioid cells were present and the changes resembled a tuberculous type of response; but usually (apart from the presence of the organism) there was nothing to suggest a specific type of reaction. FIG. 2A shows a portion of the wall of one of the abscess cavities.

The central part of the nodule usually contained chlamydospores of the fungus. These cells reached their maximum size in 2–6 weeks. In some of the older nodules (two months or more) the fungus cells were disintegrating or had been completely absorbed. FIG. 2D, *b*, shows a chlamydospore beginning to disintegrate; the outer wall is separating from the inner wall, forming blisters on the surface of the cell. Sometimes, when mounted in potassium hydroxide, the outer wall separates completely from the inner, as in FIG. 3B. This figure recalls Jellison's illustration of *H. parvum* in the lungs of a rock rabbit (**14**), and suggests that chlamydospores of *H. parvum* disintegrate in wild animals in the same way as in experimental animals. A later stage of disintegration, in which the outer wall has completely disappeared, is shown in FIG. 2D, *d*.

Chlamydospores of Montana strain 14 were only 6–9 μ in diameter after six days in the animal and closely resembled those seen in the lungs of Arizona rodents. FIG. 2E, shows these small spores in giant cells. For comparison, spores of Alberta strain 8 are

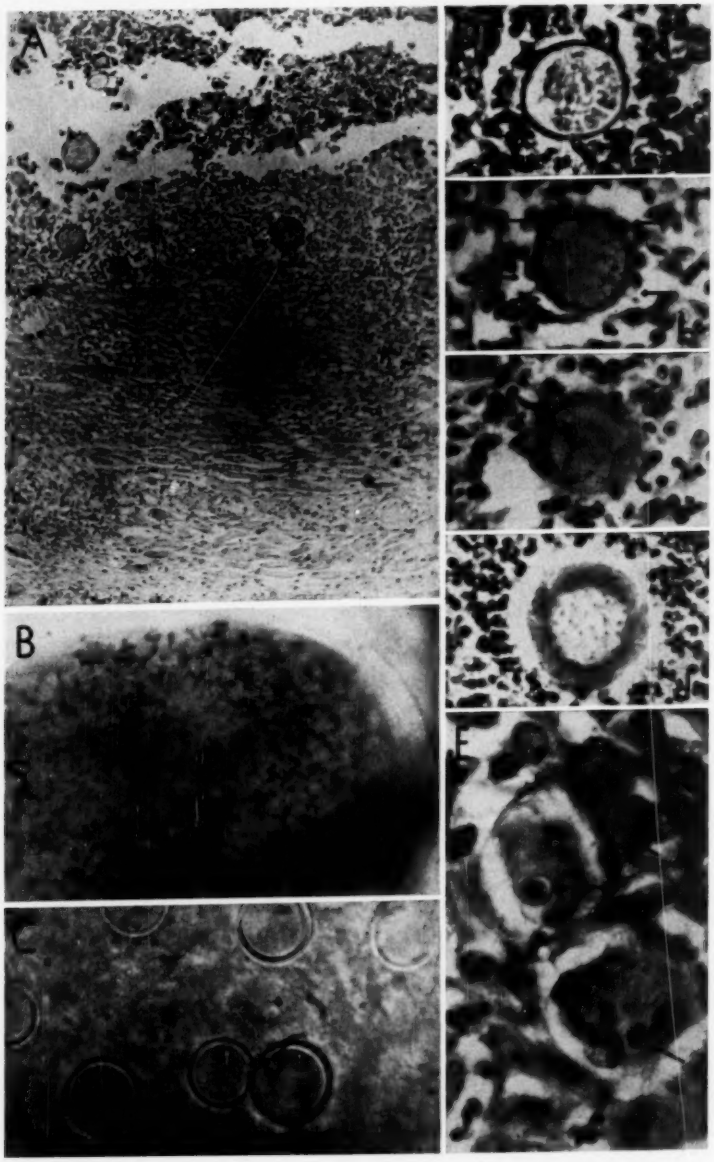

FIG. 2.

shown after the same length of time, and at the same magnification in FIG. 2C.

Spores of other northern strains were similar to those seen in the lungs of naturally infected Alberta and Montana animals. They were generally smaller, however. In one animal the maximum diameter was only 55 μ after two and one-half months. The largest cells seen in any experimental animal were 175 μ.

In one series of four guinea pigs injected with the same spore suspension (Alberta strain 5), all developed typical nodules on both sides of the abdomen within ten days. In three of the pigs the nodules disappeared after only two months. Microscopic examination at this time showed that most of the chlamydospores had disintegrated and were absorbed. In the fourth animal, both nodules remained at their maximum size (about 10 mm. in diameter) for seven months. When the animal was killed at this time the nodule contained many large chlamydospores which proved viable when cultured.

Southern Strains. Of 11 animals (8 guinea pigs and 3 mice) inoculated with southern strains, only two pigs developed nodules; moreover these disappeared in less than a month. In comparison with northern strains, spores of Arizona and Texas strains were rapidly absorbed by the animal. Usually, spores had disappeared from the site of injection in as short a time as ten days.

Generally speaking, the condition of chlamydospores in animals appeared to depend upon three factors; the time elapsed since injection, the strain used, and the resistance of the individual animal.

No evidence of internal disease was found in any of the animals

FIG. 2. *H. parvum* in experimental animals. A. Portion of an abscess of one month's duration, with chlamydospores of an Alberta strain. B. Portion of a three-week-old chlamydospore of an Alberta strain (cell-wall at right) showing nuclei. C. Chlamydospores from nodule six days after injection of an Alberta strain, mounted in Amann's fluid. D. Stages in disintegration of chlamydospores: *a*, normal cell; *b*, outer wall separating and forming "blisters" (arrows point to them); *c*, outer wall almost dissolved except for blisters (surface view); *d*, after three months, inner wall greatly thickened and irregular, protoplast disorganized. E. Montana strain 14 after 6 days, showing intracellular chlamydospores and one extracellular (compare size with C). Magnification: A. $\times 75$; B, C and E. $\times 750$; D. $\times 335$.

inoculated subcutaneously. Neither was *Haplosporangium* obtained from the lungs, liver or spleen when these organs were cultured from five animals. Cultures from subcutaneous nodules, however, produced an abundant growth of *H. parvum* in pure culture. (Chlamydospores from nodules germinate by the extrusion of several germ tubes.)

It now seems established that *H. parvum* does not multiply in the host, or cause a progressive disease (**2, 8, 15**). The presence of chlamydospores in the lung results usually in a slight, nonspecific type of reaction. Further, the fungus cells in the lung are probably destroyed in time. We may consider *H. parvum* a feebly pathogenic and probably accidental parasite in the lungs of small mammals. The parasitic cells are extremely large and easily seen. If it were not for this, *H. parvum* would be listed as a contaminant along with certain species of *Penicillium* and *Beauveria* which can also be regularly isolated from rodent lungs.

DISCUSSION

When the strains were compared with respect to five differential characteristics they separated readily into three groups.

Arizona and Texas strains: colony type, cottony; relative growth rate, 26 (mm. in 10 days); relative spore size, 3.2 μ; chlamydospores formed in culture at 40°, in animals 10–40 μ; not usually forming nodules when injected subcutaneously.

Montana and Idaho strains: colony type, floccose; relative growth rate, 18; relative spore size, 3–6 μ; chlamydospores formed in culture at 37° and 40°, in animals to 400 μ; forming nodules when injected subcutaneously.

Alberta strains: colony type, granular; relative growth rate, 16; relative spore size, 3.8 μ; chlamydospores formed in culture at 37° and 40°, in animals to 300 μ; forming nodules when injected subcutaneously.

The microscopic morphology (which is described in Part II) is similar in all three groups. Other than the size differences noted above, they cannot be separated on this basis.

One strain, Montana 14, did not fit the three-group classification. It did not form typical chlamydospores in culture at either 37° or

40°; its conidia were too large for the Montana-Idaho group; its chlamydospores in experimental animals were small; and its growth rate was faster than that of the other Montana strains. This strain is intermediate between the Arizona and Montana groups in some respects, and intermediate between the Montana and Alberta groups in other respects.

It is evident that *H. parvum* could be split into either two species (northern strains and southern strains), or three species (Arizona and Texas strains, Montana and Idaho strains, and Alberta strains). However, from what is known of the distribution of this fungus, it is likely that other intermediate forms may be found in the states to the north and east of Arizona which would invalidate these species. The scattered reports from Minnesota, British Columbia, and California indicate that *H. parvum* may be very widespread in its distribution.

All strains studied form a complex which seems homogeneous enough to be covered by one specific name. The known distribution of *H. parvum* rather suggests that the three groups are ecologic units within the species which have become adapted to different climates. That the main distinguishing features are differences in degree of response to temperature, supports this idea. Alberta strains and Montana strains are sufficiently different in the sum of their characters to be recognized as forms. The Alberta-Montana strain complex and the Arizona-Texas strains are more sharply demarcated and could be given varietal distinction. The writer suggests, however, that the addition of names to the literature should be delayed until the distribution and taxonomic position of this fungus are better known.

PART II: GENERIC RELATIONSHIPS

MORPHOLOGY

Although a morphological study of *H. parvum* revealed no structural differences among the fifteen strains, it did reveal characters which have a bearing on the taxonomy of the species as a whole.

When *H. parvum* is grown in petri dishes on corn-meal agar the fertile hyphae are at first erect or ascending (FIG. 1B). They

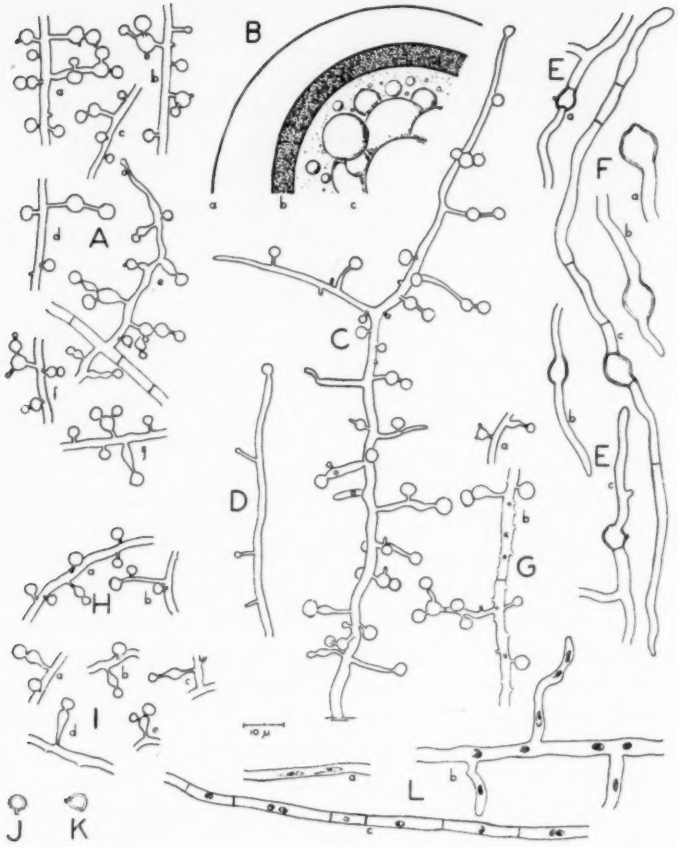

FIG. 3. Hyphae and spores of *H. parvum*. A and C. Various strains showing secondary conidia (hyphae with septa not shown are in air). B. Part of a chlamydospore (in potassium hydroxide) that has developed in a guinea pig for 5 months: *a*, outer wall, which has separated completely from the inner wall *b*; *c*, protoplast containing globules which stain with Sudan IV. D. Young fertile hypha. E. *a* to *c*, spores of a Montana strain germinating at room temperature; *a* and *c*, spores with point of attachment to conidiophore still visible. F. *a* to *c*, spores of an Arizona strain germinating at 37° C. G. *a* and *b*, old fertile hyphae with spores detached, showing remnants of conidiophores. H. Hyphae with slender conidiophores. I. With ampulliform conidiophores. J and K. Conidia with remnants of conidiophores. K. Showing point of attachment to a secondary conidium. L. Hyphal nuclei; *a* and *b*, young hyphae; *c*, older hypha. Magnification of all drawings, including B, × 600.

arise from the subsurface mycelium or from trailing hyphal ropes. They are either simple, or branched at right angles to give a tree-like habit (FIG. 3C). These fertile hyphae are not highly specialized and do not differ markedly from the vegetative mycelium; in fact there is a gradation from completely sterile hyphae to those which bear many spores.

The spores themselves are borne on short conidiophores which are usually very narrow (FIG. 3H). However, some of the conidiophores are swollen distally as in FIG. 3I. After the conidium is partially mature, one or more secondary conidiophores often grow out from it. These in turn produce secondary conidia (FIG. 3A). This process may be continued, and occasionally chains of three or rarely four conidia are formed. In some cultures secondary conidia are very numerous (FIG. 1C).

The conidia are liberated by fracture or dissolution of the conidiophore wall at a point between the basal septum of the conidium and the parent hypha. This leaves scars along the fertile hyphae (FIG. 3G). No septum is visible in spent conidiophores and the open end is apparently plugged with a refractile substance (FIG. 4B, *d*). The ragged end of the broken conidiophore can often be seen at the base of the freed conidium (FIG. 3J and 4B, *c*). Conidia which have produced secondary spores can be seen to have two or more points of abscission (FIG. 3K).

Conidia of *H. parvum* have a double wall. There is a thin, refractile outer layer which is often spiny, and a thicker, less refractile inner layer (FIG. 4B, *c*). The tremendous enlargement of the spores in animals, and their subsequent disintegration makes the two layers easily visible (FIG. 3B and 2D, *b*).

Conidia placed on Sabouraud's agar at room temperature begin to germinate in 14 hours by the formation of a single germ tube. Later a second germ tube grows from the opposite side of the spore (FIG. 3E).

The nuclei of *H. parvum* are fairly evenly spaced along the hyphae and are never seen two abreast (FIG. 3L, *a* and *b*). In older hyphae and in the conidia there are one or two nuclei per cell (FIG. 3L, *c* and 1E). In both northern and southern strains, chlamydospores formed in culture become multinucleate as they

enlarge (FIG. 1F). The large chlamydospores of Alberta and Montana strains in animals contained thousands of nuclei (FIG. 2B).

DISCUSSION

Relationship with Haplosporangium Thaxter. Thaxter (21) described the genus *Haplosporangium* as similar to *Mortierella* Coemans, but differing in the possession of prostrate, highly specialized fertile hyphae (FIG. 4A, *b*). *H. parvum* does not possess

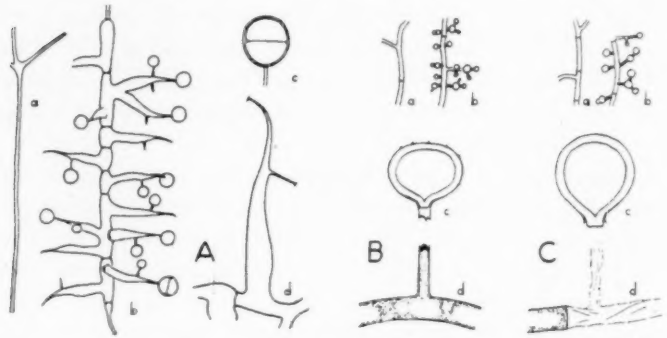

FIG. 4. Three fungi compared. A. *H. bisporale*, after Thaxter (21); B. *H. parvum*; C. *B. dermatitidis*. *a*, vegetative hyphae; *b*, fertile hyphae; *c*, spores; *d*, sporophores after release of spores, showing collarettes (in A), protoplasmic plug (in B), and dissolving walls (in C). Drawings *a* and *b* are at the same magnification for each of the three species.

such specialized hyphae. It differs also from Thaxter's two species in having stipitate, often catenate conidia. Furthermore, Thaxter's drawings (FIG. 4A, *d*) show that in his species (as in *Mortierella*) there is a collarette left at the tip of the sporangiophore after release of the spores. In *H. parvum* the spores are released by fracture or dissolution of the sporophore itself; consequently no collarette can be seen, and the spores often have remnants of the conidiophore attached to the stipitate portion (FIG. 4B, *c* and *d*). In *H. parvum* the fertile hyphae are erect and the conidiophores do not taper. In the other three species of *Haplosporangium* (*H. bisporale* Thaxt., *H. decipiens* Thaxt., and *H. lignicola* Martin (20)) the fertile hyphae are prostrate and the sporangiophores taper.

Therefore, *H. parvum* does not belong in the genus *Haplosporangium* as constituted by Thaxter, and should be removed as soon as a more suitable placement can be made.

Relationship with Coccidioides. Another possible point of attachment with the Phycomycetes is through *Coccidioides immitis* Rixford and Gilchrist. Earlier (**12**), it was considered that *H. parvum* was probably related to *Coccidioides* for the following reasons. 1. The two fungi were found associated in the same hosts and locations. 2. In his first series of wild rodents, Emmons observed endospores in the lungs of animals from which only *H. parvum* was obtained in culture. 3. Parasitic cells of the two fungi were indistinguishable at some stages of their development. 4. Skin-test antigens of the two fungi may produce cross reactions.

The following recently discovered facts argue against a close relationship between *Haplosporangium parvum* and *Coccidioides immitis*. 1. *C. immitis* is probably restricted to certain semi-arid areas (**19**), while *H. parvum*, as we now know, is very cosmopolitan in its range and hosts (**15**). 2. Ashburn and Emmons (**2**) were unable to find endospores in 200 animals infected with pure cultures of *Haplosporangium*. In Montana and Alberta, where *Coccidioides* does not occur, endospores have not been found in wild animals, nor has endosporulation been observed in experimental animals inoculated with these strains. There is still no evidence to dispute Dowding's statements (**8**) that "Chlamydospores of *Haplosporangium parvum* do not bud. They do not proliferate in any way. They merely enlarge." (In the host.) 3. The mold fungi pathogenic for man and animals present a reduced morphology in the host, many occurring as single cells when parasitic (e.g., *Histoplasma, Blastomyces, Sporotrichum, Phialophora, Hormodendrum,* and *Candida*). That *H. parvum* and *C. immitis* should both occur as single spherical cells under these conditions is not surprising, and does not necessarily imply relationship. 4. Although infection with *H. parvum* produces a sensitivity detectable with a coccidioidin skin-test (**11**), Howell (**13**) has shown that such non-specific reactions are probably due to high antigen concentrations. Bearing in mind the differences in the life-cycles, morphology, and distribution of the two fungi, we are brought to believe that any similarities are coincidental with their parasitism and do not denote relationship.

Relationship with the Phycomycetes. It has been suggested that the conidia of *H. parvum* are monosporous sporangioles (**12**). Their double spore wall is not a safe criterion of this since many higher fungi show this feature. *Sclerotinia vaccinii* (**16**), *Thielaviopsis basicola* (**16**), many rusts (**1**, FIG. 25 *a, f, h,* and *o;* **6**, Figs. 6–10), *Scopulariopsis brevicaulis* and *Blastomyces dermatitidis* (writer's observations) have double-walled spores. The smut *Mykosyrinx cissi* has spores with two cells which are enclosed by a common outer wall, which at maturity ruptures, freeing the spores (**3**).

If *H. parvum* is removed from the genus *Haplosporangium* and is not related to *C. immitis,* then it has no obvious connection with the Phycomycetes. In the absence of a sexual stage it would then be placed in the family Moniliaceae of the Fungi Imperfecti.

Relationship with Blastomyces and Histoplasma. Fertile hyphae of *H. parvum, Blastomyces dermatitides* Gilchrist & Stokes and *Histoplasma capsulatum* Darling are almost indistinguishable. The demonstration of secondary conidia in *H. parvum, B. dermatitidis* (FIG. 1D), and *H. capsulatum* (**10**) provides another point of similarity among fungi that already have been considered a natural group (**8, 9**), and sets them apart from other imperfect forms. Of the three, *H. capsulatum* is distinct with its tuberculate macroconidia, while *H. parvum* and *B. dermatitidis* are more closely akin (FIG. 4, B, C).

Whether or not to place *H. parvum* and *B. dermatitidis* in the same genus depends on the taxonomic weight given their parasitic phases. (It should be remembered here that *B. dermatitidis* and *B. brasiliensis* (Splendore) Conant and Howell are wrongly placed in the genus *Blastomyces* Cost. and Roll. (**5**).) An acceptable classification of *Haplosporangium parvum* and other dimorphic fungi must await a better understanding of relationships in the Moniliaceae as a whole.

SUMMARY

1. Fifteen strains of *Haplosporangium parvum* derived from Arizona, Texas, Montana, Idaho and Alberta animals were studied comparatively.

2. Their colonial morphology reflects to some extent their geographic origin.

3. Northern strains are slower growing at room temperature than southern strains, their spores are slightly larger, they form chlamydospores at a lower temperature. Unlike most southern strains they form nodules when injected subcutaneously into guinea pigs and mice.

4. One Montana strain was like northern strains in some particulars and like southern strains in others.

5. All strains are similar in microscopic morphology.

6. The differences among strains do not justify splitting *H. parvum* into two or more species.

7. The possession of erect fertile hyphae and chains of conidia will necessitate the exclusion of *H. parvum* from the genus *Haplosporangium* Thaxter.

8. *H. parvum* is probably not related to *Coccidioides*, or any other Phycomycete. Its closest affinity is with *Blastomyces dermatitidis*.

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THE EFFECT OF NUTRITION ON THE SPORULATION OF MELANCONIUM FULIGINEUM IN CULTURE[1,2]

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(WITH 2 FIGURES)

Nutrition is one of the most important factors which influence sporulation in fungi. It is necessary to know the nutritional requirements before the influence of other factors can be properly evaluated. The source of carbon, source of nitrogen, vitamins, micro elements and concentration of nutrients may affect sporulation as well as growth. Since fungi vary greatly in their requirements for sporulation, it is necessary to make extensive studies of a number of species before drawing general conclusions regarding the conditions which lead to sporulation.

Preliminary experiments showed that *Melanconium fuligineum* readily produced abundant spores in culture and that sporulation was affected by nutritional factors.[4] This fungus was then selected as the test organism for more intensive studies of these factors. The purpose of this paper is to report the results of these studies.

MATERIALS AND METHODS

The isolate of the fungus used in these experiments was obtained from a rotted grape at Morgantown, West Virginia.

Natural media prepared from grape juice, whole wheat, and potatoes were used in some preliminary experiments. A glucose-casein hydrolysate medium prepared according to Leonian and

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 437.

[2] This paper is based on work conducted for Camp Detrick, under Contract No. W-15-035-CE-167 with West Virginia University.

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[4] In an abstract of the preliminary work this fungus was mistakenly identified as *Guignardia bidwellii*. (See Amer. Jour. Bot. **36**: 811, 1949.)

Lilly (4) was used for studying the vitamin requirements of this fungus. The following synthetic basal medium was used to determine the best sources of carbon and nitrogen for sporulation:

Carbon source	20	g.
Nitrogen source to furnish	0.425	g. N
KH_2PO_4	1	g.
$MgSO_4 \cdot 7H_2O$	0.5	g.
Agar (when used)	20	g.
Fe^{+++}	0.2	mg.
Zn^{++}	0.2	mg.
Mn^{++}	0.1	mg.
Thiamine hydrochloride	50	μg.
Distilled water to make	1000	ml.

The reaction of all media, except as noted, was adjusted to pH 5.0 before sterilization. Media were autoclaved 15 minutes at 15 pounds steam pressure. Although the use of agar introduced an uncertainty in the composition of the medium, no evidence was obtained which indicated any nutritional effect due to the agar.

Petri dishes were used for agar media. For liquid media, the culture vessels used were 250 ml. Erlenmeyer flasks. Each culture vessel received 25 ml. of medium. Unless otherwise stated, the cultures were incubated at 25 ± 1° C. in a culture room equipped with a time clock which operated daylight fluorescent lamps giving 12 hours of light and 12 hours of darkness each day.

After preliminary experiments, the use of a heavy spore inoculum which covered the agar surface was adopted, as it proved to be the best method of inoculation for rapid sporulation. Methods of inoculation and their effects on sporulation are discussed in another paper (7). One milliliter of spore suspension was used to inoculate each plate. The plates were then gently agitated to disperse the spores uniformly over the surface of the agar.

A haemocytometer was used to determine the approximate maximum numbers of spores produced per culture, but this method was soon abandoned because of the time and labor involved. Since the blackness of the cultures was found to be correlated with the relative abundance of spores, visual observation was then used to rank the cultures on an arbitrary scale. The highest rank of 5 represents the greatest number of spores (approximately 2 to 3 billion per culture). The sporulation rating as determined by two inde-

pendent observers usually agreed, and never differed more than one unit. The appearance of cultures having sporulation ranks of 1, 3, and 5 is shown in FIG. 1.

The amount of mycelium produced under various conditions was determined by harvesting and weighing after drying to constant weight. With cultures on agar media the following procedure was used: twenty milliliters of distilled water were added to each culture and the agar was then melted in an autoclave. The mycelium was collected from the melted agar, washed with warm water,

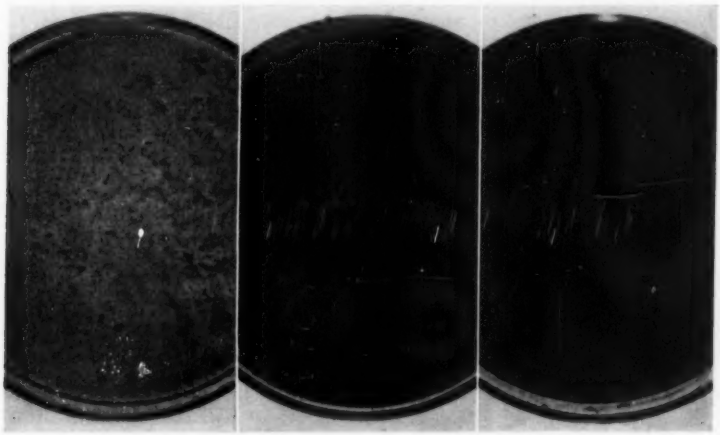

FIG. 1. Appearance of cultures having sporulation ranks of 1, 3 and 5 (left to right) as determined by visual observation.

filtered and dried to constant weight. This method of determining the amount of growth on agar media has been used by Fries (3) and Day and Hervey (2).

At least four plates were used for each medium tested. This number was increased to 16 in those experiments where the mycelium was harvested. All experiments were repeated at least twice.

EXPERIMENTAL RESULTS

Vitamin requirements. A preliminary investigation revealed that this fungus failed to grow on liquid glucose-casein hydrolysate medium which had been treated to render it essentially free from vita-

TABLE I

THE RELATIVE AMOUNT OF SPORULATION AS A FUNCTION OF THE THIAMINE CONCENTRATION ON MALTOSE-AMMONIUM TARTRATE MEDIUM. READINGS MADE AFTER 4 DAYS INCUBATION

µg. Thiamine/1000 ml.	Sporulation rank
0.0	0
0.78	1
1.56	1
3.125	2
6.25	3
12.50	3
25.00	5
50.00	5

mins. Further testing showed this fungus to be deficient for thiamine. The response of this fungus to graded amounts of thiamine is shown in FIG. 2. Sporulation occurred only in the flasks containing the higher concentrations of thiamine.

The effect of thiamine concentration upon the production of spores was investigated using the basal medium which contained

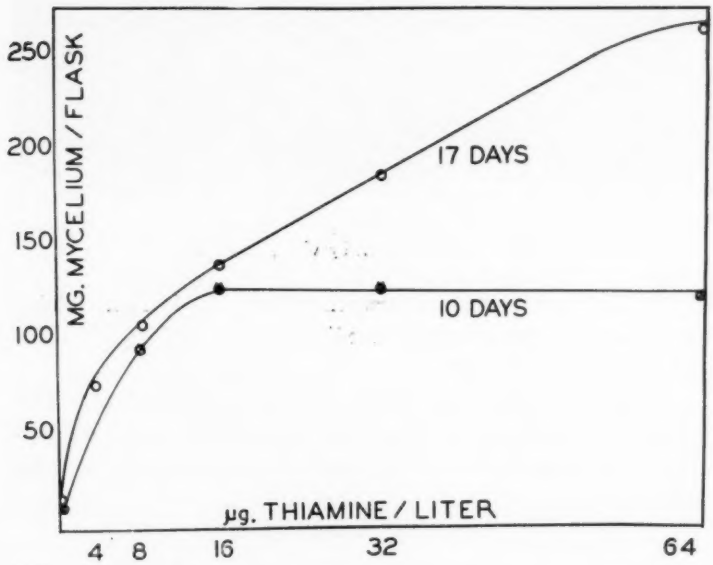

FIG. 2. The effect of increasing amounts of thiamine upon the amount of vegetative growth on 25 ml. casein-hydrolysate medium at 10 and 17 days.

maltose and ammonium tartrate. This medium was solidified with purified agar. Varying amounts of thiamine were used and the abundance of sporulation, estimated by visual observations, was found to depend upon the thiamine concentration of the medium. The essential data are given in TABLE I. Inasmuch as 50 micrograms of thiamine per liter yielded maximum sporulation, this concentration was used in later experiments. The reduced amount of sporulation obtained when low concentrations of thiamine were used may have been due to the utilization of this sub-optimal amount primarily for the production of vegetative mycelium.

Concentration of nutrients. The effect of different concentrations of nutrients on the amount of sporulation was investigated

TABLE II

THE EFFECT OF NUTRIENT CONCENTRATION ON THE AMOUNT OF SPORULATION ON GLUCOSE-CASEIN HYDROLYSATE MEDIUM SOLIDIFIED BY AGAR. TIME OF INCUBATION 4 DAYS

Concentration of nutrients	Sporulation rank
2×	5
1×	5
½×	4
¼×	3
⅛×	2
1/16×	1

using glucose-casein hydrolysate medium solidified with agar. Thiamine was added to all media at the rate of 50 micrograms per liter. The data are presented in TABLE II. Under these conditions, a rather high concentration of nutrients (25 to 50 grams of sugar per liter) favored sporulation.

Other experiments were made with media which contained 16 times the normal concentration of nutrients. The production of spores on 16 × maltose-ammonium tartrate medium began on the fifth day, whereas spores formed within 24 hours on 1 × medium.

Carbon and nitrogen sources. Different carbon and nitrogen sources are utilized by fungi with varying degrees of efficiency. The rate and amount of growth is a measure of the efficiency. Sporulation is also affected by these variables. A given combination of carbon and nitrogen sources may be excellent for both growth and sporulation, or poor for growth and excellent for sporulation, or *vice versa*. The influence of eight carbon sources

TABLE III

SPORULATION RANK OF CULTURES ON 64 COMBINATIONS OF CARBON AND NITROGEN SOURCES. READINGS TAKEN AT 3 AND 8 DAYS

Carbon sources	Nitrogen sources									
	Days of incubation	Ammonium tartrate	Casein hydrolysate	Asparagine	Potassium nitrate	Glutamic acid	Urea	Ammonium sulfate	Glycine	Average
Maltose	3	5	5	2	4	2	4	3	1	3.2
	8	5	5	4	4	5	5	5	4	4.6
D-Fructose	3	4	4	3	3	3	3	2	0	2.8
	8	5	4	5	3	4	4	1	3	4.0
D-Glucose	3	3	3	3	3	0	3	2	0	2.3
	8	4	4	5	3	4	3	3	4	3.8
Sucrose	3	4	4	3	3	2	0	2	0	2.3
	8	5	4	4	3	4	3	4	4	3.9
D-Galactose	3	3	3	3	2	3	2	3	0	2.4
	8	4	4	4	2	4	3	3	3	3.4
Cellobiose	3	3	2	2	3	3	3	2	1	2.4
	8	4	4	3	3	5	3	4	4	3.8
Potato starch	3	2	2	2	3	2	2	2	1	2.0
	8	2	2	2	3	3	2	2	2	2.3
Lactose	3	1	0	0	0	0	0	1	0	0.3
	8	1	2	1	1	1	1	2	1	1.3
Average	3	3.1	2.9	2.3	2.6	1.9	2.1	2.1	0.4	
	8	3.8	3.6	3.5	2.8	3.4	3.0	3.4	3.1	

and eight nitrogen sources on sporulation of this fungus was investigated by using all possible combinations, 64 in all. At the same time, a portion of each medium was reserved for growth studies in liquid media. The purpose was to determine which carbon-nitrogen combination gave maximum sporulation in the shortest time, and whether there was any correlation between growth and sporulation. This experiment was repeated twice with closely agreeing results. Since the data are too extensive to present in detail, only the amounts of sporulation at 3 and 8 days after inoculation are given in TABLE III.

It is evident from the data in TABLE III that the time required for maximum sporulation as well as the amount of sporulation de-

pends upon the carbon and nitrogen sources used. Lactose and potato starch were poor carbon sources irrespective of the nitrogen source used. The effect of glycine was noteworthy. This compound was a fair to good nitrogen source, but it retarded maximum sporulation. Potassium nitrate and urea were also poor nitrogen sources. Ammonium tartrate, on the other hand, supported early maximum sporulation. Maltose and ammonium tartrate appeared to be the best combination, both from the standpoint of numbers of spores produced and the time required to produce them. The superiority of maltose and ammonium tartrate was maintained when these compounds were used in combination with other nitrogen and carbon sources, as is shown by the average sporulation values in TABLE III.

A parallel experiment was made using liquid media, containing the same carbon and nitrogen sources as before. The cultures grown on liquid media produced spores, and the value of these combinations of carbon and nitrogen sources was much the same as when the media were solidified with agar. It was noted that the better media for sporulation were those which gave the most rapid rate of early growth.

It was observed that the spores germinated at different rates on different media. It was desirable, therefore, to determine if there was any relation between the rate of germination of the spores used as inoculum and the rate and amount of sporulation of the cultures. It was of interest to determine at the same time if there was a relation between growth and sporulation. Combinations of carbon sources and four nitrogen sources were used in this experiment, 16 media in all. The media were solidified with agar, the weight of mycelium and amount of sporulation determined as described in the section on methods. Microscopic examinations were made daily until it was feasible to rank sporulation by visual comparison. A portion of these data is presented in TABLE IV.

Germination of spores used as inoculum and early growth of the mycelium were more rapid on media containing maltose than those containing glucose, sucrose or cellobiose. The production of spores on the young mycelium on maltose-ammonium tartrate medium began within 24 hours. On most other media the beginning of

sporulation was correlated with rapid germination of inoculum and rapid early growth of mycelium.

The rate of growth of mycelium is likewise an important factor in determining the time of maximum sporulation on any given medium. Maximum sporulation occurs near the time the weight of the mycelium is at the maximum (TABLE IV). Exhaustion of one or more nutrients may be assumed to occur at this time. Qualitative tests for reducing sugar in maltose-ammonium tartrate medium indicated that only a trace of sugar remained after four days of incubation.

TABLE IV

RATE OF SPORE GERMINATION, GROWTH OF MYCELIUM AND SPORULATION RANK ON DIFFERENT MEDIA

Media	Rate of germination	Days of incubation	Mg. mycelium	Sporulation rank
Maltose-ammonium tartrate	rapid	3	130	4
		5	167	5
		8	157	5
Maltose-glycine	rapid	3	89	1
		5	233	4
		8	158	4
Glucose-ammonium tartrate	slow	3	51	2
		5	189	4
		8	163	4
Glucose-glycine	slow	3	11	1
		5	138	2
		8	197	4

Temperature, pH and light. The effect of the initial pH of maltose-ammonium tartrate medium was investigated. This fungus sporulated well over a wide range of pH values. The optimum initial pH was near 5.0. Many spores were produced when the initial pH was as low as 3.0. Likewise, when this fungus was cultured on media containing ammonium sulfate as a source of nitrogen the pH fell as low as 2.0, yet sporulation took place.

Cultures sporulated equally well in light or darkness. Sporulation was somewhat more rapid at 28° than at 20° C. Higher and lower temperatures were not investigated.

DISCUSSION

The influence of vitamins on asexual reproduction of vitamin-deficient fungi has been noted by several investigators (Magrou and Mariat, **5**; Marsh and Bollenbacker, **6**). The effect of vitamins on asexual reproduction may be less clear cut than on sexual reproduction (Barnett and Lilly, **1**). It was possible to obtain a trace of vegetative growth on *M. fuligineum* on agar media containing extremely low concentrations of thiamine, without sporulation. On liquid medium the effect of low thiamine concentrations in preventing sporulation was more easily demonstrated. This may have been due to the more extensive development of submerged mycelium, and possibly to the destruction of a portion of the thiamine as a result of growth processes.

The influence of carbon and nitrogen sources on growth and sporulation has been studied by many investigators. While these effects may be ascribed to the metabolic potentialities of the organisms, the molecular configuration and composition of the carbon and nitrogen sources, such generalizations are too broad to be of much assistance in understanding the specific causes of superiority or inferiority.

In these experiments we have found that one of the prerequisites for rapid sporulation and early maximum production of spores is rapid germination of the spores used as inoculum and rapid early growth of the mycelium. Spore germination and the rate of growth are influenced both by the source of nitrogen and source of carbon in the medium.

On the basis of these experiments we would recommend the use of maltose-ammonium tartrate or maltose-casein hydrolysate medium for the production of quantities of spores of *M. fuligineum* in the shortest time. For this purpose maltose was decidedly superior to the more commonly used glucose or sucrose. Likewise, ammonium tartrate and casein hydrolysate were superior nitrogen sources when compared to the more commonly used nitrate and asparagine. We do not mean to imply that these same media would necessarily yield the greatest abundance of spores of other fungi. On the contrary, the evidence indicates that species of fungi vary greatly in their nutritional requirements for sporulation. It

will be necessary to study the nutritional and environmental requirements of many species of fungi before any general conclusions can be drawn.

SUMMARY

M. juligineum is deficient for thiamine. Limited vegetative growth is possible in concentrations of thiamine so low that spores fail to form. Sixty-four combinations of carbon and nitrogen sources were tested for suitability as sporulation media. Maltose was the best carbon source tested, while ammonium tartrate and casein hydrolysate were the best sources of nitrogen. Lactose was poorly utilized. Growth was poor and only a trace of sporulation resulted on media containing this sugar. Rapid and abundant sporulation is correlated with rapid germination, and rapid early vegetative growth. The most favorable initial pH for sporulation was about 5.0. Sporulation occurred at pH values as low as 2.0. Sporulation was only slightly less rapid at 20° than at 28° C. Light did not appear to have any influence on sporulation.

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THE EFFECT OF CYCLOHEXIMIDE[1] ON THE SPOROPHYTE OF ALLOMYCES ARBUSCULA

ALMA J. WHIFFEN

(WITH 1 FIGURE)

The spontaneous transition in *Allomyces arbuscula* from a sporophytic to a gametophytic state without the intervention of the resistant sporangium and its meiospores has been previously reported by Sörgel (**1937**). What appears to be a similar vegetative transformation of a sporophytic plant into a gametophytic plant has been consistently effected by treatment of the sporophyte of *A. arbuscula* with the antibiotic cycloheximide. The purpose of this paper is to describe and discuss the nature of the transformation induced by cycloheximide.

MATERIALS AND METHODS

The strain of *Allomyces arbuscula* used in these studies was isolated from Texan soil and designated number *196*. Its life cycle is typical of the sub-genus *Euallomyces* in which the transition from the sporophyte to gametophyte is normally effected by the germination of the resistant sporangium at which time meiosis occurs (Emerson and Wilson, **1949**). Initially, a genetically homogenous clone of strain *196* was obtained by germinating a resistant sporangium and isolating a single meiospore (for explanation of terminology see Emerson, **1950**), which gave rise to a gametophyte. The gametophyte was selfed and a single zygote was isolated. The sporophyte thus obtained was maintained in culture and used for all subsequent studies. The culture medium in all experiments was P.D.Y. agar of the following composition: bacto-peptone 1 gram, Difco yeast extract 1 gram, glucose 10 grams, bacto-agar 20 grams and distilled water 1 liter.

Aqueous solutions were prepared of the following antibiotics: crystalline cycloheximide, streptomycin sulphate (800 units per

[1] Cycloheximide is the generic name of the antibiotic for which The Upjohn Company has adopted the trademark, "Acti-dione."

milligram), streptothricin sulphate (400 units per milligram), neomycin sulphate (200 units per milligram) and endomycin (1480 units per milligram). Streptomycin and neomycin are not inhibitory to the growth of fungi. Streptothricin, cycloheximide, and endomycin (Gottlieb *et al.*, **1951**) all inhibit the growth of fungi to some degree. Suitable dilutions of the antibiotics were made as needed by adding the antibiotic solution to melted P.D.Y. agar that was allowed to harden in petri dishes. Transfers of the fungus were made by means of a disc, four millimeters in diameter, cut from the margin of a four-day-old colony. The mycelium in this disc of inoculum lacked resting sporangia so that the possibility of a gametophyte arising from the chance germination of a resistant sporangium was obviated. Surfaces of the agar plates were sufficiently dried before use to prevent the discharge of any spores. All plates were incubated at 24° C.

EXPERIMENTAL RESULTS

The minimal concentration in micrograms per milliliter of each of the five antibiotics required to inhibit completely the growth of the sporophyte of *A. arbuscula 196* was found to be as follows: cycloheximide 0.8, streptomycin > 1000, streptothricin 1000, neomycin > 1000, and endomycin 4.0. Growth of the sporophyte was partially inhibited by 0.4 to 0.1 microgram of cycloheximide per milliliter. In seven days, growth on agar containing 0.4 microgram of cycloheximide per milliliter was 35 per cent of the control lacking cycloheximide, 60 per cent of the control on 0.2 microgram per milliliter, and 80 per cent of the control on 0.1 microgram per milliliter. Germination of mitospores (see Emerson, **1950**) was prevented by 0.2 microgram of cycloheximide per milliliter.

After the sporophyte had been exposed to cycloheximide in concentrations of 0.4 or 0.2 microgram per milliliter for ten to fourteen days, during which period of time it grew slowly, there were apparent in portions of mycelium proximal to the disc of inoculum numerous orange-colored structures that resembled male gametangia. This phenomenon was first observed with cycloheximide. Subsequently, the ability of neomycin, streptomycin, streptothricin, and endomycin to effect this transformation was investigated. Endo-

mycin was more effective than streptothricin, streptomycin, and neomycin in causing production of orange-colored reproductive structures. Colored structures were observed in three out of ten colonies growing on agar containing 2.0 micrograms of endomycin per milliliter. They were observed in only one of ten colonies exposed to a concentration of 1000 micrograms of streptomycin per milliliter or to 100 micrograms of neomycin per milliliter. No colored structures were seen, however, in ten colonies grown on 1000 micrograms of neomycin per milliliter or on 500 micrograms of streptothricin per milliliter. A larger number of trials might have revealed a greater effectiveness of these antibiotics than here recorded. Cycloheximide was much more active than endomycin, neomycin, streptomycin, and streptothricin in effecting the production of colored structures. Within a fourteen-day period of exposure to cycloheximide colored structures appeared on ten to twenty hyphae in seven out of ten colonies. In twenty control colonies a single orange-colored structure was observed.

Further studies were made of the effectiveness of cycloheximide in causing the production of orange-colored structures. In order to determine whether or not a germinating mitospore could be transformed *in toto* into what would appear to be a gametophyte, a suspension of mitospores was dispensed in 0.05 milliliter drops on agar plates containing 0.1 microgram of cycloheximide per milliliter. The mitospores germinated slowly, requiring three days to reach a stage of development attained by the control spores in one day. Reproductive structures formed on the control plants within two days but five days were required for the development of reproductive structures on the cycloheximide-inhibited plants. After six days of incubation, it was apparent that four of approximately ninety plants had produced orange-colored structures on every branch of the young plant. A number of other plants bore orange-colored structures on one or two branches only. A plant that was completely gametophytic in appearance was photographed both in color and in black and white. The black and white photograph is reproduced as FIG. 1C in which the orange-colored structures have been outlined with black ink. A concentration of 0.05 microgram per milliliter also yielded plants of gametophytic appearance. No col-

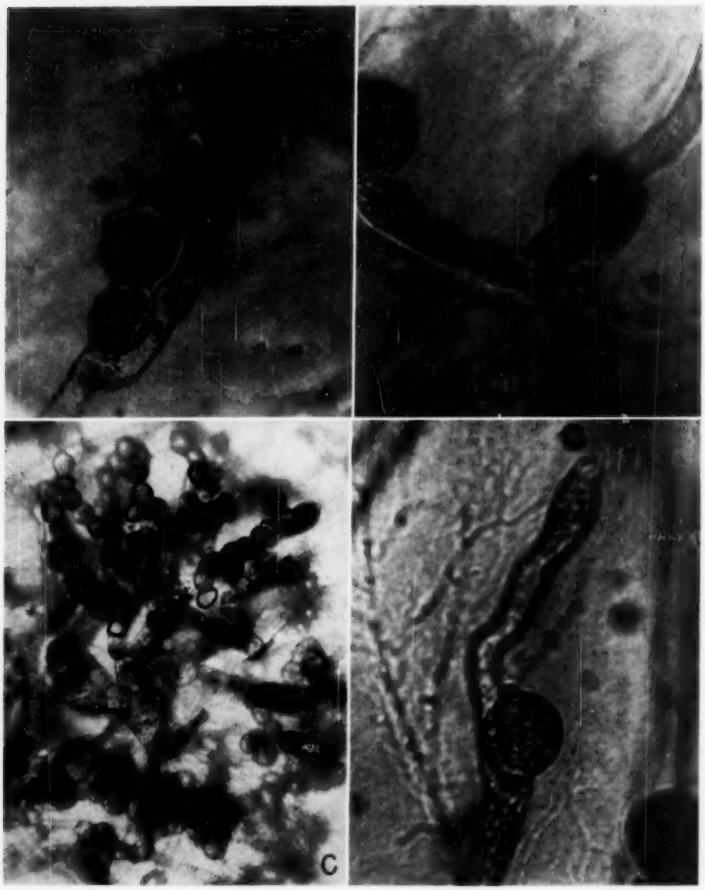

FIG. 1. *Allomyces arbuscula*. A. Two pairs of male and female gametangia from a gametophyte. × 290. B. A zoosporangium from an untreated sporophyte. × 290. C. A gametophytic plant derived from a mitospore (zoospore) on agar containing cycloheximide; male gametangia are outlined in ink. × 74. D. An orange-colored structure (male gametangium) borne on a sporophyte treated with cycloheximide. × 370. (The photographs were made by Mr. Norman Drake of The Upjohn Company.)

ored structures were observed among approximately two hundred plants from control mitospores.

The most obvious interpretation of the orange-colored structures produced on the sporophyte is that they are actually male gametangia and that some or all of the colorless reproductive structures associated with them are female gametangia. Results of experiments now to be reported would indicate the above interpretation to be correct. From sporophytic colonies exposed to cycloheximide for two weeks, there were dissected out singly six hyphae bearing orange-colored structures. Each hypha was transplanted to cycloheximide-free agar and allowed to develop into a colony which was examined microscopically after four days of incubation. At that time isolation number one had the appearance of a normal gametophyte. Pairs of colorless and colored reproductive structures alternated on the distal portions of the hyphae in an hypogynous arrangement typical of the gametophyte of *A. arbuscula*, a condition that will be designated henceforth by the letter G. Isolation six, which produced both zoosporangia and resting sporangia, had the appearance of a typical sporophyte, a condition to be referred to by the letter S. The colonies of the other four isolations consisted entirely or partially of mycelium exhibiting characteristics of both G and S. This mixture of G and S will be called GS. In the colonies of GS there were resting sporangia and numerous orange-colored structures which frequently were borne singly at the point of origin of a hyphal branch in the same manner as zoosporangia on the sporophyte. In the gametophyte it is usual for this position to be occupied by a pair rather than by a single reproductive structure. The typical G situation is shown in FIG. 1A and the typical S situation in FIG. 1B. FIG. 1D is a photograph of a single orange-colored structure from a GS sector. A photograph in color was made of this same structure but is not reproduced here. Isolations two and five initially consisted of sectors of G and GS while isolations three and four were entirely GS.

Subsequently each isolation was transferred serially three times at seven-day intervals. At the end of each seven-day period of growth the colonies were examined and characterized. Inoculum for each transfer was cut from the margin of the colony. In those

colonies in which sectors of GS and S or G were co-existant, the inoculum was taken from the GS sector. Isolation one, originally G, remained G and isolation six remained S through all transfers. After the second transfer, isolation three ceased producing orange-colored structures and assumed a S appearance. The G characteristics of isolations two, four, and five gradually increased through the first two transfers so that after the third transfer all three were completely G. The changes during serial transfer of all six isolations are summarized in TABLE I.

TABLE I

CHARACTERISTICS OF SIX ISOLATIONS FROM CYCLOHEXIMIDE-TREATED SPOROPHYTE

Isolation No.	Original isolation	Transfer 1	Transfer 2	Transfer 3
1	G	G	G	G
2	G+GS	G+GS	G+GS	G
3	GS	S+GS	S	S
4	GS	G+GS	G+GS	G
5	G+GS	GS	G+GS	G
6	S	S	S	S

G + GS or S + GS indicates sectoring. See text for explanation of symbols.

In order to investigate the possible gametophytic nature of the orange-colored structures and associated colorless structures, several of these structures were dissected singly from isolation number four subsequent to transferring it for the third time. Each structure was isolated in a drop of water, into which swimming spores were discharged. These spores were examined under the microscope and transferred in a capillary pipette to the surface of an agar plate. In this way putative gametes were obtained from three orange-colored and two colorless structures. Those obtained from the colored structures were orange-pigmented and corresponded in size to male gametes and those from the colorless structure lacked pigment and were the same size as female gametes. None of the putative gametes germinated but when the small and large spores were mixed together fusion in pairs was observed and from the zygotes sporophytes were obtained. Therefore, in behavior and appearance the reproductive structures borne by the cycloheximide-induced gametophyte could not be distinguished from those borne by a gametophyte arising from a meiospore.

It was of further interest to determine whether or not cycloheximide would induce the formation of gametangia on the sporophyte of *Allomyces anomala*, a species that is characterized by the lack of a gametophyte in its life cycle. A culture of *A. anomala* was obtained from Dr. J. N. Couch. Its growth was completely inhibited by a concentration of 0.5 microgram of cycloheximide per milliliter of agar and partially inhibited by 0.2 microgram per milliliter. In each of three trials ten transfers were made to agar containing 0.2 microgram of cycloheximide per milliliter. At the end of two weeks of incubation a microscopic examination for orange-colored structures was made and none was found.

DISCUSSION AND CONCLUSIONS

The evidence obtained from the experimental work described above seems to justify the conclusion that the orange-colored structures produced by the sporophyte under the influence of cycloheximide are male gametangia and that the colorless structures paired with them are female gametangia. The manner in which cycloheximide effects this transformation is obscure. If the gametophytic state in *A. arbuscula* is determined by the haploid chromosome number—and the cytological evidence obtained by Emerson and Wilson (**1949**) of meiosis in the germinating resting sporangium strongly supports such a belief—then it would appear that cycloheximide interferes with mitotic division in such a way as to cause separation of the chromosomes into two groups without splitting. Cytological evidence of "reductional groupings" in the sense of Huskins (**1948**) would be difficult to obtain because of the small size of the mitotic figures in this fungus as in fungi in general. It would be of interest to compare meiotic figures in a sporophyte derived from fusion of gametes from a cycloheximide-induced gametophyte with the meiotic figures in a germinating resting sporangium from a sporophytic plant of strain *196* that had not been treated with cycloheximide.

If cycloheximide does effect a somatic or vegetative reduction division, it might be expected that some of the hyphae that bear gametangia on a cycloheximide-treated sporophyte might contain mixtures of haploid and diploid nuclei in varying proportions.

Then, if chromosome number determines "gametophyticness" and "sporophyticness," the ratio of haploid to diploid nuclei might possibly determine whether the colony growing out from a single hypha bearing cycloheximide-induced gametangia will have the characteristics of a gametophyte, or of a sporophyte or of both. A gradual shift during growth of the fungus in the ratio of diploid to haploid nuclei toward dominance of either the haploid or the diploid might explain the observed change from the GS condition to G or S condition in isolations three and five described above.

It should be emphasized that cycloheximide is not unique in its ability to induce the production of gametangia on the sporophyte of *A. arbuscula*. As shown, endomycin possesses this ability to a lesser degree and if a search were made, there might be found compounds more active than cycloheximide. These probably would differ greatly from cycloheximide and endomycin in chemical structure. Furthermore, it should be pointed out that in inducing the formation of gametangia on the sporophyte, cycloheximide and other effective compounds probably are acting only to intensify a tendency already present in the organism. This contention is borne out by the fact that during the course of this study a male gametangium was observed on a sporophyte not exposed to cycloheximide and in earlier studies on the nutrition of strain *196* a few such orange-colored structures had previously been seen and isolated. Finally, it would appear that the production of gametangia on the sporophyte can be effected readily only if a gametophyte occurs regularly in the life cycle as in *A. arbuscula* but not in *A. anomala*.

The transformation from sporophyte to gametophyte, which cycloheximide induces, appears to be the same as found by Sörgel (**1937**) to occur spontaneously in his strain 40 of *A. Kniepii* (*A. arbuscula*). Sörgel's sectors of "mischhyphen," bearing mixtures of gametangia and zoosporangia and resting sporangia, correspond closely to the GS sectors described above. On the basis of differential measurements of nuclear volumes, Sörgel concluded that the "mischhyphen" contained a mixture of haploid and diploid nuclei and he attributed the mixture of sporophytic and gametophytic characteristics to the presence of both haploid and diploid nuclei

in the mycelium. This interpretation by Sörgel and the hypothesis proposed above to explain the means by which cycloheximide effects the transformation from sporophyte to gametophyte are in agreement. As pointed out by Emerson (**1941**), the correctness of the conclusion that haploid and diploid nuclei may be found within the same coenocyte can be ascertained only through cytological investigation. The need for such cytological studies, therefore, is obvious.

SUMMARY

When the sporophyte of *Allomyces arbuscula* was grown on agar containing a concentration of cycloheximide that partially inhibited growth, orange-colored reproductive structures developed on portions of the sporophyte. A few zoospores (mitospores), when allowed to germinate in the presence of cycloheximide, gave rise to plants having the appearance of a gametophyte. The antibiotics endomycin, streptomycin, streptothricin, and neomycin were either ineffective or much less effective than cycloheximide in inducing the formation of orange-colored structures.

The swimming spores discharged by the paired colored and colorless structures correspond in behavior and size to male and female gametes. It is concluded that the colored reproductive structures and associated colorless structures are male and female gametangia whose formation on the sporophyte is induced by the action of cycloheximide. When sporophytic hyphae bearing male and female gametangia are isolated, they may give rise to colonies which at first display a mixture of sporophytic and gametophytic characteristics and which subsequently become either completely gametophytic or sporophytic in appearance. As a possible explanation of this behavior it is suggested that the isolated hypha from which the colony was derived contained a mixture of haploid and diploid nuclei and in time one kind became dominant over the other. Such an explanation assumes that chromosome number determines "sporophyticness" or "gametophyticness."

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COCCOSPORA AGRICOLA GODDARD, ITS SPECIFIC STATUS, RELATIONSHIPS, AND CELLULOLYTIC ACTIVITY

W. Lawrence White and Mary H. Downing

(with 7 figures)

OBJECTIVE

The purpose of this paper is threefold: (1) to re-establish the identity of the species *Coccospora agricola,* which has been more or less submerged and forgotten since it was described by H. N. Goddard in 1913; (2) to present certain evidence and conclusions concerning its natural phylogenetic affinities; and (3) to consider its cellulolytic activity, especially in relation to allied forms. It was through a study of cellulose-destroying fungi that the species first came to our attention.

INTRODUCTION AND REVIEW

The genus *Coccospora* Wallroth has not been subjected to any modern investigation. Its six or seven species are supposed to form sporodochia on plant substrata. It is considered to be a genus of the Tuberculariaceae.

The single species *Coccospora agricola* Goddard, with which this paper deals, is a soil-inhabiting form. On agar media it produces an effuse growth, bearing spores on short side branches in a cottony mycelium. It definitely is Moniliaceous. It has in common with the other species of *Coccospora* large globose or subglobose spores, which are 1-celled, hyaline or nearly so, and which have smooth, or slightly surface-wrinkled, unusually thick walls.

Aside from this elementary morphology there is little or no information on such fungi. Perhaps if other species of *Coccospora* were grown on agar media they too would produce the same effuse type of sporulation which is produced by *C. agricola.* Whether or not *C. agricola* and any or all of the other species of the genus are closely related phylogenetically will be determined only after further investigation on the morphology and physiology of all of the species.

The status of the genus *Coccospora* itself needs to be re-examined, especially in relation to *Sphaerosporium* Schw. The latter has nomenclatural priority and rests on a firm foundation, with the common wood-inhabiting *S. lignatile* Schw. as the type. The type species of *Coccospora, C. aurantiaca* Wallr., is of doubtful identity. It may or may not be the same species as that on which *Sphaerosporium* is based. It is not improbable that *Coccospora* will have to be referred to synonymy under *Sphaerosporium* or else discarded and the species distributed in *Sphaerosporium* and other genera.

It is evident, and will be more so after discussion further on in this paper, that nomenclatural readjustment eventually will be required for *Coccospora agricola* and many of the similar or related species of fungi. It will not be attempted here, however, for it is our conviction that the practice of name-changing in general has been taken too lightly, that it has proceeded at a rate out of all proportion to the rate of gaining new fundamental knowledge on which to base a sounder nomenclature. It is our belief that science will lose nothing if the names of such fungi as we are dealing with here are left alone for a few years until we learn more about the organisms themselves.

The species, *Coccospora agricola,* assuming that it is not buried in the literature under a still older name, was discovered by H. N. Goddard (**1913**) in garden soil in Michigan. He isolated it only once. The object of his study was to determine whether or not soil fungi were capable of fixing nitrogen. He obtained negative results for this isolate as well as for those of the several other species which he studied. He described and illustrated the species on the basis of his single isolate, but no evidence can be found that he preserved a culture or specimen. Traaen (**1914**) of Norway, in setting up his Dematiaceous genus *Humicola,* noted Goddard's *Coccospora,* but believed it to be generically distinct from his two proposed species of *Humicola* because of its thicker walled aleuriospores and lack of phialospores.

The only information on the biology of the species is that provided by Jensen (**1931a, 1931b**) in a study of the activities of the soil fungi in England and Denmark. Jensen found it to be extreme

in its intolerance of acidity and tolerance of alkalinity. He found that addition of cellulose to both acid and alkaline soils caused a multiplication of cellulolytic fungi but that totally different floras developed. The fungi developing in neutral and alkaline soil consisted of a very limited number of species, all of which were present only in very small numbers in the original soil. One of these he believed to be *Coccospora agricola,* but he did not claim certainty of identification. It proved in pure culture tests to be among the very strongest of the cellulose-decomposing fungi that were encountered. Jensen informs us (personal communication) that no culture or specimen of what he supposed to be *Coccospora agricola* was preserved. It is, however, our personal opinion that he actually did have Goddard's species.

In the same year Saccardo (**1931**, p. 633) merely presented a translation of the original description. Recently Gilman (**1945**, p. 365) excluded it from his keys and descriptions of soil fungi because of insufficient data concerning its position and occurrence.

Inquiries addressed to the curators of several herbaria and culture collections in this country and abroad have brought replies to the effect that there were no specimens or cultures on file under Goddard's binomial and that the species was unknown. It appears then that Jensen is the only worker who has identified anything with this species. It is pertinent to note also that no material has been discovered from such likely sources as cultures reported in the literature under "*Monotospora* sp.," or "*Humicola* sp."

CULTURAL CHARACTERS

One dried specimen and three cultures are here referred to *Coccospora agricola,* as follows: QM *51j,* on tentage taken in Territory of Hawaii in 1944; QM *336* (= Covert *8,* Harvard *931*), isolated at University of Pennsylvania December 1945 by Scott V. Covert from peat collected in Michigan; QM *337* (= Covert *19,* Harvard *336*), isolated at University of Pennsylvania December 1945 by Scott V. Covert from peat collected in Michigan; QM *991* (= Ajello *442,* Harvard *231*), isolated at Knoxville, Tennessee, November 1948 by Libero Ajello as a contaminant in a test tube that had been inoculated with the spleen of a rat.

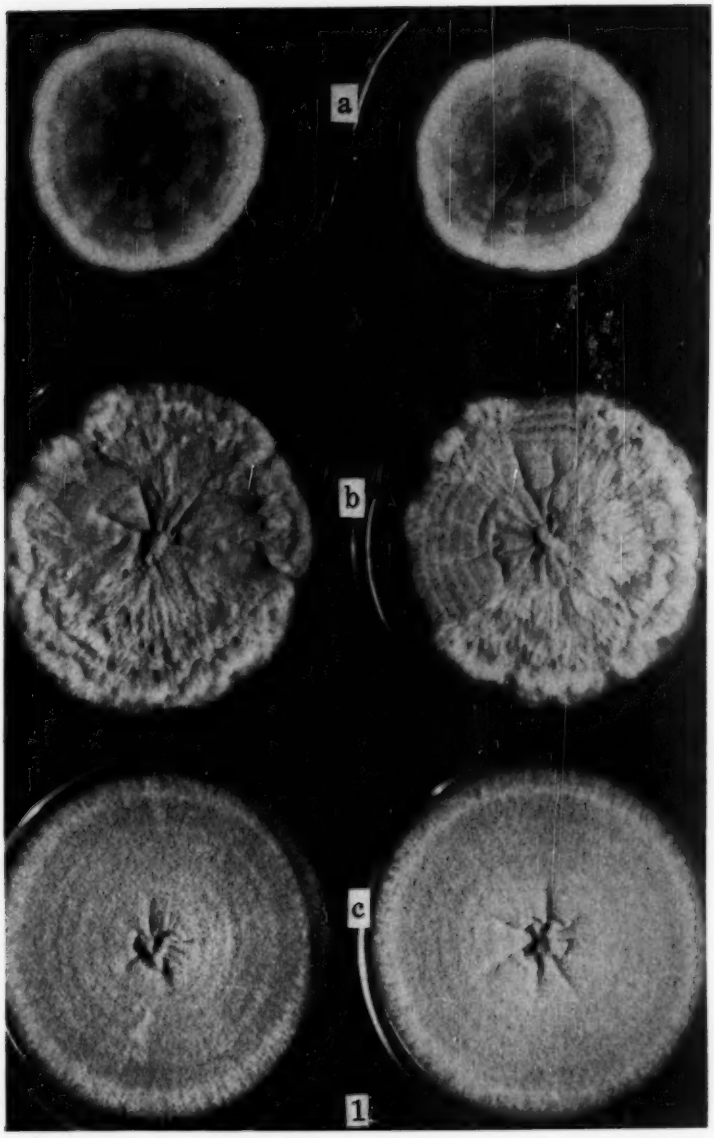

FIG. 1.

No. QM *51j* consisted of a small amount of pale ochraceous, hyphomycetous growth on a sample of deteriorated tentage. Several organisms were sporulating on the sample and several additional forms were obtained through cultural procedures. Most prominent on the sample was *Memnoniella echinata*. Some *Gliomastix convoluta* was also present. Both are strong cellulose destroyers. The *Coccospora* was closely associated with these but nothing can be said concerning any interaction. The *Coccospora* was not obtained in culture. Nor was any special effort made to obtain it, since critical surface examination of the sample was not made at the time of culturing. Spores mounted in KOH solution were globose or nearly so, golden yellow with the color apparently in the walls, variable in diameter, 13–22 μ, the content scarcely granular, the wall 3–3.5 μ thick and entirely smooth. No tendency for the wall-layers to become separated in the mounting fluid was noticed. It is with some hesitation that this is referred to *C. agricola*.

Gross characters of cultures. Cultures on the usual agar media grow rapidly and sporulate quickly and copiously. The mycelium is white or whitish at first, but with sporulation the colony becomes uniformly buff. In FIG. 1 is shown duplicate plates of the three cultures, QM *336* (FIG. 1A), QM *337* (FIG. 1B), and QM *991* (FIG. 1C), grown in parallel series on potato dextrose agar as indicated in the legend. These were started by transferring bits of mycelial growth from a similar agar culture. The colonies become "Tilleul-Buff" (Ridgeway) by the third or fourth day. QM *337* (FIG. 1B) and *991* (FIG. 1C) remain that color permanently. In QM *336* (FIG. 1A), however, there soon appear numerous sterile unbranched hair-like structures (FIG. 5), at first in concentric rings in the older central area (FIG. 1A), gradually becoming more numerous until at about the end of a month they impart a medium gray *Alternaria*-like aspect to the entire colony. Such hairs also appear occasionally in faint concentric rings in old cul-

FIG. 1. *Coccospora agricola*. A, QM *336;* B, QM *337;* C, QM *991*. Grown in parallel series under room conditions on potato dextrose agar (20 g. dext., 20 g. agar, water from 400 g. potatoes, made up to 1 liter), 35 cc. of the medium per dish. × 0.8. (Photo by Frank White, Dept. Biology, Harvard Univ.)

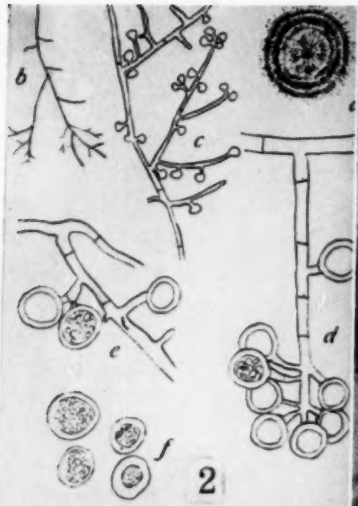

FIG. 4.—*Coccospora agricola*, n. sp.: *a*, habit sketch; *b*, branched hypha, ×80; *c*, hyphae bearing conidia on simple side branches, ×180; *d*, clustered conidiophores, ×380; *e*, simple conidiophores bearing conidia, ×380; *f*, conidia (chlamydospores), ×380.

FIGS. 2–7.

tures of QM *337* but never in such prominence as to impart their dark coloration to the colony. They have never been observed in *991*. Certain other differences are apparent in FIG. 1. QM *337* is characterized by sectors which produce an overgrowth and others in which this is absent. Heavy sporulation is present on the mycelium comprising the overgrowth as well as on that underneath. Where the overgrowth is absent the surface of the colony is much the same as that of QM *991* (FIG. 1C). The cultures have not been compared in an extensive range of media. During the period of approximately five years that two of them have been carried in stock, however, they have been grown in plate culture and photographed several times. These records present evidence of extreme plasticity, even on more or less the same medium. One photo of *336* shows no visible evidence of the hair-like growths after two months and one of *337* shows the growth developed in deep conspicuous concentric rings.

Microscopic characters. Aside from the presence or absence of the dark hairs, no differences have been noted in the three cultures. Spores from the petri plates shown in FIG. 1, mounted in water, gave the following measurements:

Culture	Range	Average of 70
QM *336*	10–17μ	13.9μ
QM *337*	11–21μ	15.1μ
QM *991*	10–18μ	14.9μ

Under a good dissecting microscope they may be seen individually as they are borne rather copiously, singly, or more often in small

FIGS. 2–7. *Coccospora agricola*. 2. Goddard's original drawings reproduced from the Botanical Gazette. 3. Conidiophores with conidia attached (compare with Goddard's *d*), from culture grown 10 days under room conditions on Czapek's agar medium, mounted in 7 percent KOH solution and stained with phloxine, × 1000. 4. Conidia from same culture, mounted in 7 percent KOH, unstained. 5. Basal portion of one of the sterile dark hair-like filaments which appears among the sporulating hyphae, from a 30-day-old culture on commercial Bacto-Difco potato dextrose agar (2 per cent dext.), mounted in lacto-phenol, unstained, × 2000. 6. Four spores, perhaps slightly immature, borne on short side branches of a hypha, from 7-day-old culture on P.D.A. as above, mounted directly in aqueous phloxine, × 2000. 7. Spores showing loosened outer layer of wall, from culture grown 14 days on Czapek's agar, mounted in 7 percent KOH to which a drop of aqueous phloxine was added, × 1000. (Photos 3–5 from culture QM *336;* 6–7 from QM *337;* 3, 4, and 7 by the junior author; 2 by Frank White, and 5, 6 by Paul Brown, both of Harvard University.)

clusters, on the rather loosely arranged hyphae. Thus viewed, they appear globose, subhyaline, smooth, glistening. In microscope preparations they are hyaline, thick walled—up to 3.5 μ, with the walls perhaps slightly reticulate (FIGS. 3 & 4). In either water or KOH preparations, but especially in the latter, what appears to be an outer membrane commonly separates from the main wall (FIG. 7), ruptures, and may tend to partially disappear.

Since Goddard did not preserve any material, identification of the current cultures can be made only by comparison with his original descriptions and illustrations. His illustrations are therefore reproduced in FIG. 2. For convenience the original description is quoted here:

"*Coccospora agricola*, n. sp.—*Mycelium orbicular*, at first white with a tufted center and radiate border, becoming slightly zoned with concentric grooves and ridges, turning pinkish brown especially inside, finally forming a powdery pinkish brown surface with age; reverse side weakly orange. *Hyphae* very little branched, septate, 4–6 μ broad, hyaline. *Conidiophores* little differentiated, consisting of short side branches, each bearing a single spore at the end, or sometimes forming racemose clusters; side branches 12–30 μ long, generally septate. *Conidia* (chlamydospores) large, thick-walled, mostly globular, very persistent, not being set free in water, 16–25 μ in diameter; membrane hyaline, 2–3 μ thick; contents highly granular and faintly brownish—fig. 4.

"The large, thick-walled, persistent chlamydospores, and the simple method of fructification, were the most strikingly characteristic features of this fungus."

COCCOSPORA AGRICOLA AND THE GENUS HUMICOLA

A consideration of all characters, both morphological and physiological, convince us that *Coccospora agricola* has extremely close natural affinities with the two species upon which Traaen established his genus *Humicola*, i.e., *H. fuscoatra* Traaen (type) and *H. grisea* Traaen. All three species occur naturally as soil fungi. With the exception of *C. agricola*, QM *51j*, none of them have ever been observed in any recognizable state on any substratum under field conditions. Many cultures of *H. fuscoatra* have been obtained from deteriorated cotton fabrics, perhaps many or most of the samples having had soil contact, but the organism does not sporulate there so that it can be studied directly, in contrast, for example, with the habit of *Memnoniella echinata*. They all grow relatively rapidly and sporulate readily at room temperatures on the common

agar culture media, including filter paper and cotton fabric on a mineral salts medium. They rank among the strongest cellulose-destroyers known. Jensen's finding of a high pH preference for *Coccospora agricola* corresponds with similar findings in our own laboratory for *Humicola fuscoatra*. All three species have globose spores borne singly on short side branches. The walls are relatively thick and have a "double contour" with the outer wall, under certain conditions, tending to separate from the inner. The two species of *Humicola* are gray or dark as sporulation advances whereas *Coccospora agricola* is pale buff. The spores of *Humicola fuscoatra* are smaller than those of *Coccospora agricola*, have walls that are less thickened, and are colored in mass although when viewed singly they are subhyaline. Those of *Humicola grisea* are about the same size as those of *Coccospora agricola* but are deeply colored.

A fourth species which we would consider here is *Monotospora lanuginosa* (Griff. & Maubl.) Mason. It is similar to *Humicola fuscoatra* but has smaller spores, is thermophilic, and is not cellulolytic. It occurs in compost piles.

We plan to treat the remaining three of these four closely related species in future papers. For the present we prefer to build around the genus *Humicola*. We find it difficult to accept the thesis that *Humicola grisea* is synonymous with *Monotospora toruloides* Corda, which would make *Humicola* Traaen a synonym of *Monotospora* Corda.

CELLULOLYTIC ACTIVITY

The form with which we are mainly concerned here, *Coccospora agricola*, had been shown to be strongly cellulolytic by Covert (Ph.D. thesis, University of Pennsylvania) from whom we obtained two of our cultures. The similar findings of Jensen are also recalled at this point. During the past few years *Humicola fuscoatra*, which is the form commonly encountered by those working on fabric decay, has come to be generally known as a strongly cellulolytic species. To our knowledge *H. grisea* has not previously been tested. Reese (Ph.D. thesis, Pennsylvania State College) had found *Monotospora lanuginosa* to be non-cellulolytic. So while there was at hand considerable cumulative information, it seemed

TABLE 1

CELLULOLYTIC ACTIVITY OF *Humicola*-LIKE ORGANISMS ON 12 OZ. GREY COTTON DUCK

Culture number	Percent loss in tensile strength	Final pH of liquid	Cx activity mg./ml./hr.	Growth index	Appearance of growth	
					In test	On 0.5 per cent P.D.A. inoculum tube
Coccospora agricola QM *336*	94	6.8	.54	4	Grey, covering strip, heavier at surface of liquid	Cream colored
QM *337*	100	7.0	.40	3	White, covering most of strip, heavier at surface of liquid	Cream colored
QM *991*	100	6.8	.30	2	Heavy greyish white, at surface of liquid only	Cream colored
Humicola fuscoatra QM *34e*	100	6.9	.15	3	White over dark grey, less floccose than above	Grey appressed
QM *71c*	66	6.9	.37	4	Pinkish over light grey	Pinkish tan
QM *73d*	100	7.0	.12	4	Heavy, grey over black, heavier at liquid surface	Grey over black
QM *73e*	100	6.7	.19	4	As QM *73d*	Grey over black
QM *130e*	100	6.9	.19	3	As QM *73d* but less growth	White over grey
QM *133a*	100	7.0	.11	4	As QM *73d*	White over black
QM *136c*	100	6.7	.11	4	As QM *73d*	Grey over black
QM *154e*	100	6.8	.07	4	Pinkish over black	Pink over black
QM *161*	100	6.7	.15	4	White over black	White over black
QM *162*	100	6.7	.15	4	White over black	White over black
QM *468*	100	6.7	.12	4	White over black	White over black
QM *469*	82	6.6	.07	4	Deep yellow over brownish	Deep yellow over brownish
QM *470*	100	7.0	.06	4	Deep grey over black	Grey over black
QM *473*	100	6.7	.22	3	Spotty deep grey over black, heavier at liquid surface	Grey over black
QM *997*	41	6.5	.03	0	No growth on strip, mycelium in liquid	Grey, moist appressed
QM *999*	100	6.6	.12	4	Deep grey over black, strip yellow green	White over grey
QM *1000*	100	6.9	.22	4	Deep grey over black, not floccose	Grey over black
QM *1001*	100	6.7	.29	4	White, floccose over dark grey	White over black

TABLE 1—*Continued*

Culture number	Percent loss in tensile strength	Final pH of liquid	Cx activity mg./ml./hr.	Growth index	Appearance of growth	
					In test	On 0.5 percent P.D.A. inoculum tube
Humicola fuscoatra						
QM *1002*	100	6.8	.16	4	Grey over black	White over black
QM *1003*	100	6.7	.40	4	Grey over black	Grey over black
JQMD *1017*	100			4	Grey over black	Grey over black
JQMD *1021*	100			4	White over grey	White over black
JQMD *1026*	100	6.7	.50	4	Deep pink-peach over yellow-brown	Pink over pinkish brown
JQMD *1031*	77	6.7	.04	4	Small amount, white over appressed black	Grey over black
JQMD *1045*	100	6.7	.23	4	Grey over black	Grey over black
JQMD *1052*	100	6.5	.20	4	As JQMD *1045*, more appressed	Grey over black
Humicola grisea						
QM *228*	80	5.7	.19	3	Heavy dark grey over most of fabric; black at liquid surface	Black
*QM *228*	88			3	As at 30° C.	Black
QM *542*	100	6.6	.38	4	Heavy, grey over black, heavier at liquid surface	Grey over black
QM *992*	100	6.5	.62	4	As QM *542*	Grey over black
QM *993*	100	6.7	.32	4	As QM *542*, darker	Dark grey over black
QM *994*	100	6.7	.24	4	As QM *542*, plus slight greenish grey	Dark grey over black
QM *995*	100	6.7	.24	4	As QM *994*	Dark grey over black
QM *996*	100	6.7	.05	4	As QM *994*	Dark grey over black
Monotospora lanuginosa						
QM *225*	2	6.8	.02	0	No growth	
*QM *225*	3	6.8	.00	1	Mycelium at liquid surface	Tan over grey
QM *226*	3	7.3	.00	1	Small scattered grey growth darker at liquid surface	
*QM *226*	2	7.1	.00	2	As at 30° C. but heavier	Dark grey over black
QM *227*	3	7.0	.02	0	No growth	
*QM *227*	2	7.1	.00	1	Dark mycelium at liquid surface and scattered over cloth	Dark grey over black

* Incubated at 40° C.

highly desirable to run a final set of tests on most of the cultures that had come to hand.

The activity of each isolate was tested by growing on 3 × 1 inch strips of 12 oz. grey cotton duck, the decline in tensile strength being taken as the measure of cellulolytic activity. Ten replicates were used for each culture. The strips were placed 1 each in test tubes of 18 × 150 mm. size, in which the lower half of the strip was submerged in 9 ml. of a liquid medium. The tubes were plugged with non-absorbent cotton and autoclaved at 121° C. for 15 minutes. The liquid medium was composed as follows: 1 percent yeast extract, 10 ml.; 10 percent NH_4NO_3, 10 ml.; 10 percent $MgSO_4$, 3 ml.; M/1 KH_2PO_4 buffer pH 4.5, 10 ml.; dist. H_2O, 960 ml. The pH was brought to 6.4 by the addition of 4.5 ml. of N/1 NaOH. Distilled water was added to bring the total volume to 1000 ml.

For inoculum the cultures to be tested were grown on slants of 0.5 percent potato dextrose agar under room conditions for about one month. Before using, the spore surface of each slant was wet out with 2 to 3 drops of 0.5 percent solution of Aerosol O T, then 20 ml. of sterile distilled water was added to each tube and the spores brought into suspension by agitation with the tip of the pipette. A 1 ml. spore suspension was placed by means of the pipette on each cloth strip. Two sets each of QM *225, 226, 227* and *228* were inoculated, one to be incubated at 30° C. and the other at 40° C. All others were incubated at 30° C. only. Each tube was examined for the amount and type of growth and a rough numerical value given each to indicate the relative amount present 13 days after inoculation. The strips were harvested by removing them from the liquid, washing in 50 percent ethyl alcohol for 30 minutes, rinsing in tap water, drying at room temperature, conditioning at 21° C. and 65 percent relative humidity, and breaking on the Scott Tester. Measurements of pH and tests for Cx activity were made on the liquid from each representative culture. The Cx activity is reported as the amount of reducing sugar (as glucose) produced by a filtrate acting on carboxymethylcellulose (CMC 50T) in one hour at 50° C. Activities of 0.05 mg./ml./hr. or less were considered doubtful or negative. Cx is the polysaccharase hydrolyzing the 1, 4 glucosidic linkages of the cellulose chain.

It will be noted that the various isolates tested, except those of *Monotospora lanuginosa* (inactive at both 30° C. and 40° C.), proved to be uniformly highly active in the degradation of the cotton duck, and that with two exceptions (*Humicola fuscoatra*, QM *997* and JQMD *1031*) they showed Cx activity. It is evident from the data in the two right-hand columns of the table that great variation in color of colony is exhibited among the various strains within a species.

SUMMARY

Three living cultures are identified as *Coccospora agricola*, a species described by H. N. Goddard in 1913, but which has remained an unknown entity since that time. Goddard's species is considered to be re-established. It is a soil-inhabiting species, strongly cellulolytic, and marked especially by its large, globose, hyaline spores whose walls are unusually thick. Its cultural characters, morphology, strong cellulolytic activity and soil habitat are strongly suggestive of the genus *Humicola* Traaen, which contains just two species, *H. fuscoatra* (type) and *H. grisea*. Nomenclatural readjustment is deferred pending a thorough study of a wide range of similar or related fungi.

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A NEW SPECIES OF NOCARDIA, N. FASTIDIOSA, N. SP., ISOLATED FROM A PENILE ULCER [1]

LOYAL S. SUTER

(WITH 4 FIGURES)

During 1948, in this laboratory, four strains of *Nocardia* were isolated in pure culture. Three of these strains were hardy-growing, aerobic, partially acid-fast, typical *Nocardias*. Two of these three strains were isolated from blood culture; the other was isolated from a brain abscess. These three have been tentatively identified as *Nocardia asteroides*. The fourth strain, *K 1*, with which this paper is concerned, isolated in large numbers from a penile ulcer, exhibited characteristics of such a nature that it could not be identified with any species described in Bergey's Manual, nor, to the knowledge of the author, with any other species described since the latest revision of Bergey's Manual.

ISOLATION OF THE ORGANISM, K 1

A Gram-stained preparation of scrapings made from a penile ulcer revealed numerous Gram-positive, filamentous, clubbed, pleomorphic organisms. Branches were not apparent. Material was removed from the ulcer with a wire loop and streaked upon a blood agar plate and upon a plate of Difco brain heart infusion (BHI) containing 2 percent agar. Plates were incubated 3 days in an anaerobic jar using yellow phosphorus for removing oxygen. Examination of the blood agar plate revealed 118 colonies that resembled actinomycetes and 15 staphylococcal colonies. The BHI agar plate revealed 257 colonies that resembled actinomycetes and 22 staphylococcal colonies.

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

CASE REPORT

A 35-year-old single, white mechanic was admitted to the hospital for removal of a cellular fibroma on the right thigh and for operation on a torn ligament of the knee. History revealed that the patient had had syphilis 11 years previously and typhoid 20 years previously. Serological studies for syphilis made at the time of entrance to the hospital were negative. During hospitalization the patient took leave of absence on two occasions for periods of approximately one month each. History of sexual exposure was not recorded. Three days after returning from the second leave of absence he was referred to the laboratory for examination of a penile ulcer of several days duration. The ulcer was flat and superficial, measuring approximately 1 cm. in diameter, and was covered with a small amount of grayish exudate; it was located about 2 cm. behind the corona on the dorsal aspect of the penis after retraction of the prepuce. Darkfield examination for *Treponema pallida* was negative. Gram stain revealed no organisms resembling *Hemophilus ducreyi*. The ulcer healed spontaneously in a few days. The patient stated that a few months previously he had had a similar lesion which likewise had healed spontaneously.

METHODS AND MATERIALS

In order to prevent duplication, some of the methods used in this study have been incorporated in the section devoted to the description of the organism.

Incubation of tubes at 17–20° C. was accomplished by immersing them in a battery jar into which tap water constantly flowed.

When necessary to prevent evaporation of moisture from plates or tubes, they were either wrapped in heavy waxed paper or placed in a jar containing water over which was placed a lid having an opening 1 inch in diameter.

The phosphorus jar was prepared in the following way: a cube of yellow phosphorus was placed in a beaker containing some $CaCl_2$. After ignition of the phosphorus, the beaker and its contents were transferred to a jar equipped with an air-tight lid.

Synthetic media were inoculated with 0.2 ml. of a faintly cloudy suspension in normal saline of the organisms removed from blood agar plates by scraping over the surface with a wire loop.

Gram's iodine solution used in the diastatic test was prepared as follows: iodine crystals 1 g., KI 2 g., and distilled water 300 ml.

Ziehl's carbol-fuchsin was prepared according to the procedures recommended in Pure Culture Study of Bacteria (Conn, **1946**). The decolorizing agent was 95 percent ethyl alcohol containing 3 percent by volume of concentrated HCl.

Blood agar was prepared from tryptose blood agar base to which was added 5 percent citrated human blood taken from Baxter transfusion bottles. These bottles contained 380 ml. of blood and 120 ml. of the following solution: dextrose U.S.P. 3.3 percent, sodium citrate U.S.P. 1.33 percent and citric acid U.S.P. 0.47 percent.

Carrot and potato slants were autoclaved; glycerine potato slants were prepared by dipping the slants in glycerol before autoclaving.

Fermentation media were made from nutrient broth using brom cresol purple as an indicator, and contained 1 percent carbohydrate.

Gelatin media were composed of 12.5 percent gelatin in brain heart infusion. For liquefaction studies the tubes were incubated at 37° C. and then cooled in the refrigerator for reading.

For the detection of indol the Ehrlich-Boehme test as described in the Difco Manual (**1948**) was used.

In testing for the reduction of nitrates to nitrites the sulfanilic acid and alpha-naphthylene test was employed.

The synthetic medium mineral base used was that employed by Bushnell and Haas (**1941**) and was prepared as follows: distilled water 1000 ml.; $MgSO_4$, 0.2 g.; $CaCl_2 \cdot 2H_2O$, 0.02 g.; KH_2PO_4, 1.0 g.; $(NH_4)_2SO_4$, 1.0 g.; $FeCl_3 \cdot 6H_2O$, 0.09 ml. of a saturated solution. Upon addition of the $FeCl_3$ a fine flocculent precipitate was formed which was filtered out, leaving a clear solution. The pH was adjusted to 7.0. Carbon compounds were sterilized separately and added in the following concentrations: paraffin 5 percent, agar 0.1 percent, starch, dextrose and glycerol 1 percent of each.

Other media and reagents used were as follows: Difco brain heart infusion (BHI), Difco nutrient agar, Difco nutrient broth, Difco Sabouraud maltose agar, Difco Sabouraud dextrose agar, Difco standardized gelatin, Difco tryptose blood agar base, fresh skim milk plus Merck azolitmin reagent, Difco standardized maltose,

Pfanstiehl sucrose (C.P.), Paul Lewis lactose (C.P. hydrate), refined paraffin with a melting point of 54.5–56.1° C. for histological and pharmaceutical use supplied by Standard Oil of New Jersey, granular agar-agar (U.S.P.) supplied by Fisher Scientific Company, Merck anhydrous dextrose (C.P.), Difco soluble starch, York Pharmacal Company glycerine (U.S.P.), Pine Forrest Company $MgSO_4$ crystals (U.S.P.), Merck granular $CaCl_2 \cdot 2H_2O$ (U.S.P.), Merck anhydrous KH_2PO_4 reagent, Merck $(NH_4)_2SO_4$ (A.C.S.) reagent, Mallinckrodt $FeCl_3 \cdot 6H_2O$ (A.C.S.) reagent, Merck sulfanilic acid (A.C.S.) reagent, Eastman Kodak Company alpha-naphthylamine, Merck KNO_3 (A.C.S.) reagent (NO_2 0.001 percent).

CONTROL ORGANISMS USED

The following control organisms were used in this study: *K 2*, an aerobic, hardy growing, partially acid-fast, richly branching, non-gelatin-liquefying *Nocardia* isolated from a blood culture at this hospital, tentatively identified as *Nocardia asteroides*. *K 3*, an aerobic, hardy growing, partially acid-fast, non-gelatin-liquefying, richly branching organism, isolated from a brain abscess at this hospital, tentatively identified as *Nocardia asteroides*. *K 4*, *Nocardia asteroides*.[2] *K 5*, *Actinomyces mexicanus* (*Nocardia brasilensis*).[2] *K 6*, an aerobic, hardy growing, partially acid-fast, richly branching, gelatin-liquefying *Nocardia* isolated from a blood culture at this hospital, tentatively identified as *Nocardia asteroides*. *K 7*, *Nocardia madurae*.[2]

During the first part of the study *K 5* was used as one of the controls but when a question arose as to its identity, it was discarded and *K 7* was substituted.

DESCRIPTION OF THE ORGANISM, K I

Morphology in direct smear from the penile ulcer. Many organisms were seen consisting of mycelial forms measuring up to 10 μ in length by 0.8–1 μ in diameter. They were pleomorphic in character and clubbed. There were no apparent septa, branches or nuclei (FIG. 1).

[2] Cultures kindly supplied by Dr. N. F. Conant, Duke University School of Medicine.

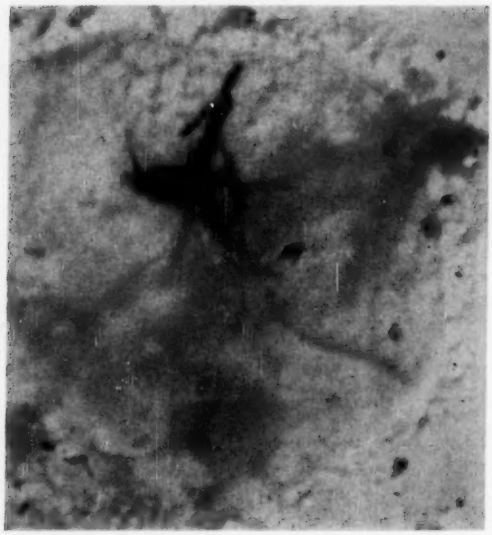

FIG. 1. *Nocardia fastidiosa.* Gram-stained preparation from penile ulcer, × 600.

Morphology of the organism seen in cultures. The organisms were similar on all media studied, showing in general a striking pleomorphism with coccoid, bacillary, and filamentous forms. Many of these were clubbed; some bore a striking resemblance to spermatozoa. Others were thick at one end and tapered down to filamentous tails. However, swollen portions were not always terminal. Portions of mycelia often were extremely small in diameter, down to 0.2 μ, and appeared tenuous. Filamentous forms were up to 25 μ in length and, not considering clubs or swollen portions, measured 0.2–1.2 μ in diameter, the average being about 0.8–1 μ (FIG. 2). Stained preparations never showed a richly branching character but an alternate type of branching was fairly easy to demonstrate after about 7 days incubation at 37° C. (FIG. 3). The coccoid forms were round, oval or drop-shaped. Neither septa nor nuclei were seen. Spores were formed in short chains within mycelial strands and were of the same diameter as the mycelial strands. Similar spores were also found singly and extracellularly.

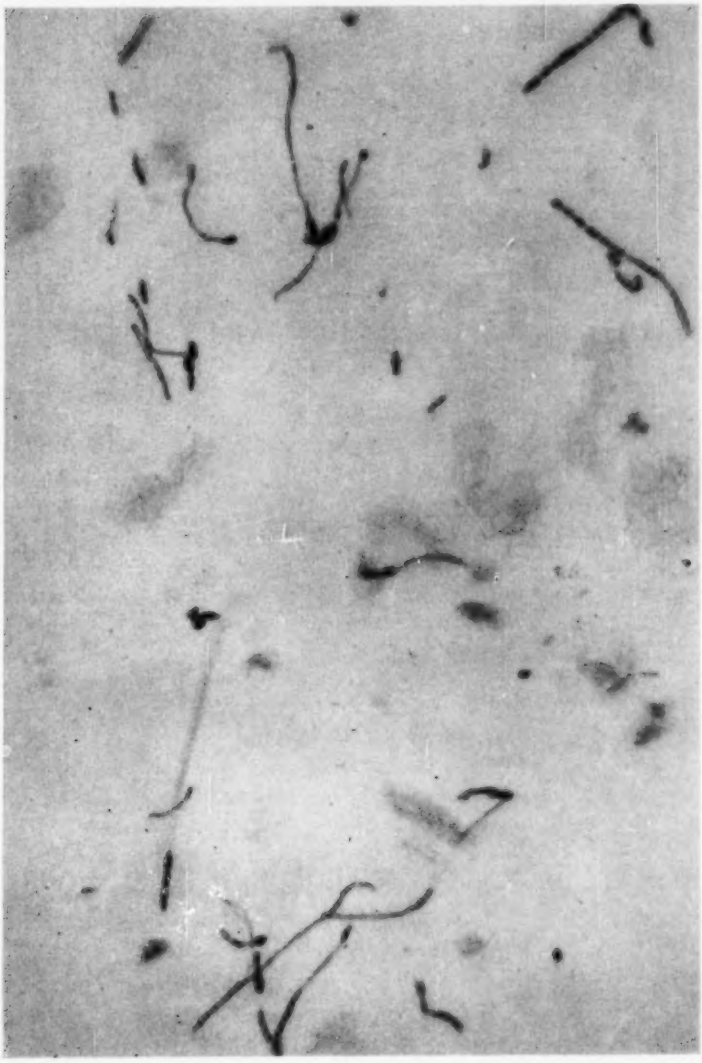

FIG. 2. *Nocardia fastidiosa*. Gram stain from culture, × 600.

Gram stain studies. The organisms in the smear made from the penile ulcer were uniformly Gram-positive. The organisms taken from cultures were Gram-positive, except that portions of mycelial strands sometimes showed a spotted or weakly staining reaction due to the presence of vacuoles or thin cytoplasm, and the tenuous portions were usually Gram-negative. Clubs, swollen portions, coccoid forms, and spores were gram positive. Numerous small granules were sometimes observed in the cytoplasm (FIG. 2).

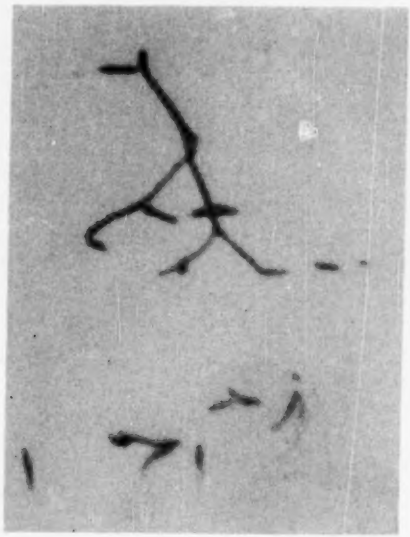

FIG. 3. *Nocardia fastidiosa.* Gram stain from culture, × 600.

Colonial morphology. Colony form of the organism was similar on all solid media (FIG. 4). Colonies were slow growing, appearing after 2 to 3 days as tiny specks which after 7 days incubation finally achieved but never exceeded a size of about 1 mm. in diameter. To the naked eye they appeared grayish white, compact, and smooth, and under low-power magnification they appeared fluffy, raised, compact at the center, and irregular and stringy at the edge due to the presence of radiating and tangled filaments. Zig-zag arrangement of elements and branches, clubs, and curls were seen at the periphery. The colonies were adherent to the

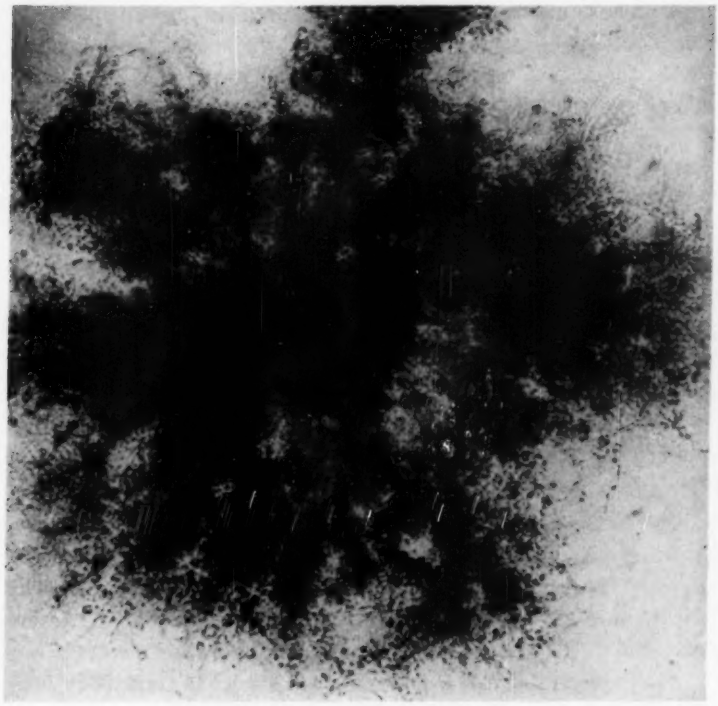

FIG. 4. *Nocardia fastidiosa.* Colony on agar plate (BHI), × 100.

medium. The top surface was dry and could be scraped off with a stiff wire loop, but neither the whole colony nor any part of it could be removed intact. On blood agar after 7 days incubation the colonies viewed by transmitted light showed a characteristic dense reddish center and a clear outer zone, both areas being very sharply defined. In fluid BHI to which 0.1 percent agar had been added, the organism grew slowly, but after 3 to 4 days at 37° C. it produced a good crop of minute glistening colonies growing down in the medium. Some of the colonies finally attained a diameter of approximately 1 mm.

Acid-fast properties. The organisms taken from cultures were partially acid-fast. In most instances the acid-fastness was confined to the spores. On one occasion mycelial strands taken from a 3-day-old blood agar plate incubated at 37° C. aerobically, re-

vealed many tiny acid-fast granules scattered throughout the cytoplasm; otherwise all mycelial forms and clubs were always non-acid-fast. Spores usually showed a good intensity of color, and they were able to withstand decolorizing for 10 minutes with 3 percent acid alcohol.

Cell refractivity. *K 1* was considered to be moderately to strongly refractive. The organisms were examined in tryptone broth in which they had been growing.

Optimum temperature. Optimum temperature for cultivation is 37° C. Good growth occurred in 3 to 5 days at 37° C. in BHI plus 0.1 per cent agar and on blood agar at 37° C. In BHI plus 0.1 percent agar at room temperature growth appeared in 14 days. There was no growth on blood agar at room temperature in 21 days. No growth appeared in 21 days at 17–20° C. using the following media: BHI plus 0.1 percent agar, blood agar slants and BHI plus 12.5 percent gelatin slants and stabs.

Oxygen requirements. The organism is a facultative anaerobe, growing equally well in the presence or absence of oxygen. A *Brewer jar* was prepared according to the directions specified by the manufacturers, using natural illuminating gas and incubating at 37° C. Blood agar plates were inoculated with the organism, *K 1*. Five aerobic *Nocardia* strains were used as controls. After 7 days incubation *K 1* showed excellent growth. However, as all aerobic *Nocardia* controls showed excellent growth it was concluded that this method was not satisfactory for achieving bacteriological anaerobiosis. *An anaerobic jar using burning yellow phosphorus* was prepared. A blood agar plate inoculated with *K 1* after 7 days incubation at 37° C. showed excellent growth. Inasmuch, however, as the five aerobic *Nocardia* controls also showed growth, though in some instances very scant, it was concluded that this method, though superior to the above, was not satisfactory for achieving strict bacteriological anaerobiosis. *Vaseline-sealed tubes of BHI.* Large tubes of BHI containing 0.1 percent agar were placed in a boiling water bath for one hour. After rapid cooling to room temperature, the tubes were inoculated with a loop of culture. Sterile vaseline at about 50° C. was immediately poured on the surface to a thickness of about 1 inch. After 7 days incu-

bation the organism, *K 1*, had shown excellent growth throughout the tube, whereas the five aerobic *Nocardia* controls all had failed to grow. It was concluded from these results that this method of obtaining anaerobiosis was superior to the other two methods described above.

Vaseline sealed thioglycollate tubes. This medium was prepared using BHI to which had been added 0.1 percent agar and *thioglycollate* supplement. Fresh media were prepared, autoclaved, and immediately cooled in a cold water bath. Tubes were inoculated as above and immediately sealed with vaseline. After 7 days incubation the organism showed an excellent growth throughout the tube, whereas all five aerobic *Nocardia* controls had failed to grow. No blue color was visible in the sealed tubes. Duplicate tubes, except for omission of the vaseline, all showed the blue zone at the top, in which all of the aerobic *Nocardia* controls grew. The organism, *K 1*, grew throughout the medium. It was concluded that this method was satisfactory for producing strict bacteriological anaerobiosis.

Proteolytic activity. The organism, *K 1*, is non-proteolytic. No odor of putrefaction was perceived in any of the cultures, gelatin was not liquefied, no growth occurred on Loeffler's serum plates, and none on coagulated human serum. Controls were all satisfactory in this study except that only two of the *Nocardias* grew on Loeffler's serum plates and this growth was very scant.

Diastatic activity. The organism, *K 1*, failed to grow in the synthetic medium to which autoclaved starch had been added in a concentration of 1 percent. Controls *K 2*, *K 3*, *K 4*, *K 6*, and *K 7* grew well in this medium. The diastatic test, performed by applying Gram's iodine to a 7-day-old streak of growth on 1 percent starch agar, was negative. Controls *K 2*, *K 3*, *K 4*, and *K 5* were also negative. *K 7* gave a positive diastatic reaction. By means of this crude test it is not possible to detect the breakdown of small amounts of starch to sugar. This problem is under investigation.

GROWTH ON CULTURE MEDIA AND BIOCHEMICAL PROPERTIES

Nutrient agar at 37° C. aerobically: results variable; sometimes failed to grow. All controls grew well.

BHI agar at 37° C. aerobically: no growth. All controls grew well.

BHI agar at 37° C. in phosphorus jar: excellent growth in 3 days.
BHI agar at room temperature aerobically: on one occasion light growth in one month.
Blood agar at 37° C. aerobically: excellent growth in 3 days.
Blood agar at 37° C. in candle jar: excellent growth in 3 days.
Blood agar at 37° C. in phosphorus jar: excellent growth in 3 days.
Blood agar at 37° C. in Brewer jar using natural illuminating gas: excellent growth in 7 days.
Blood agar at room temperature: no growth in 21 days.
Blood agar at 17–20° C.: no growth in 21 days.
BHI plus 0.1 percent agar at 37° C.: good growth in 3 days.
BHI plus 0.1 percent agar at 37° C. in phosphorus jar: good growth in 3 days.
BHI plus 0.1 percent agar at room temperature: first appearance of growth in 14 days.
BHI plus 0.1 percent agar at 17–20° C.: no growth in 28 days.
BHI plus 0.1 percent agar (9 parts) and human serum (1 part) at 37° C.: excellent growth in 3 days.
BHI at 37° C.: growth in bottom of tube.
Nutrient broth at 37° C.: growth in bottom of tube.
Nutrient broth plus 0.1 percent agar at 37° C.: no apparent growth.
Tryptose phosphate broth at 37° C.: growth in bottom of tube.
Tryptone broth at 37° C.: growth in bottom of tube.
Sabouraud maltose agar at room temperature: no growth. All controls grew well.
Sabouraud dextrose agar at room temperature: no growth. All controls grew well.
Skim milk: growth along sides of tube in seven days; formation of a soft curd in 14 days.
Litmus milk: decolorization of litmus in bottom of tube 14 days; soft curd in bottom of tube 28 days.
Carrot at 37° C.: no growth. All controls grew well.
Potato at 37° C.: no growth. All controls grew well.
Glycerine potato at 37° C.: no growth. All controls grew well.
Dextrose was fermented with formation of acid only.
Lactose, sucrose, maltose, and glycerine were not fermented.
Nitrate broth: growth questionable; test for nitrite negative. *K 2* and *K 6* were negative; *K 3, K 4, K 5,* and *K 7* were positive.
Indol production: no indol produced; all controls were negative.
Gelatin stab at 17–20° C.: no growth 28 days. Four of five controls grew.
Gelatin slant at 17–20° C.: no growth 28 days.
Synthetic mineral base: no growth. No growth of controls.
Synthetic mineral base plus 0.1 percent agar at 37° C.: no growth. Four of five controls grew.
Synthetic mineral base plus paraffin at 37° C.: no growth. Four of five controls grew.
Synthetic mineral base plus dextrose at 37° C.: no growth. All controls grew.
Synthetic mineral base plus starch at 37° C.: no growth. All controls grew.
Synthetic mineral base plus glycerine at 37° C.: no growth. All controls grew.

PATHOGENICITY FOR LABORATORY ANIMALS

No lesions were produced by the inoculation of *K 1* into rabbits, guinea pigs, and mice. A slightly cloudy suspension of *K 1* in saline was prepared by scraping growth from a blood agar plate. A rabbit, a guinea pig, and a mouse were each given 1 ml. of the suspension intraperitoneally; a rabbit and a guinea pig were each given 0.1 ml. of the suspension intracutaneously. After 40 days the animals were sacrificed; gross and microscopic examinations were negative.

DISCUSSION

The system as outlined by Waksman and Henrici (**1943**) for classifying members of the family *Actinomycetaceae* has in general been accepted in this country. This family is composed of members which, though they form a mycelial type colony, tend to fragment into bacillary and coccoid forms. The family is subdivided into the genera *Nocardia* and *Actinomyces*. The genus *Nocardia* is composed of species which are aerobic and fall into one of two categories, (1) acid-fast or partially so, and (2) non-acid-fast. The genus *Actinomyces* is composed of species which are anaerobic and non-acid-fast.

There are classified in Bergey's Manual 33 species of the genus *Nocardia*. The section devoted to the genus *Nocardia* was revised by Waksman and Henrici in May, 1943. An appendix, listing many more species, originally prepared by Waksman and Henrici in May, 1943, and further developed by Clise in August, 1945, is composed, in the words of the authors, of "species probably belonging to the genus *Nocardia*. Many are incompletely described. Some of the species listed may belong in the genus *Streptomyces*." It is assumed that additional study of the individuals listed in the appendix is indicated in order to identify them or to establish them as new species.

There are tendencies to simplify the classification of the *Nocardias*. Conant (**1948**) states that "biologically the various species are not constant in their activities or there are strain differences within a given species. It would seem, therefore, that the species concept for any one form should be flexible enough to admit great

variability without assigning new specific names for slight differences." He states (**1945**) in his discussion of *Nocardias* that cause actinomycosis that "the species in the aerobic group have been separated on such biologic activities as ability to liquefy gelatin, to peptonize milk, or to give a typrosinase reaction. It is agreed, however, that these characteristics do not remain constant, and it seems more valuable to classify these fungi by their acid-fast properties. Other biologic activities should be evaluated critically for the purpose of reducing rather than enlarging the number of species."

It is felt, from studies of the literature and from studies made in this laboratory, that acid-fastness or partial acid-fastness is a reliable character, that it should be given a prominent place as a criterion of classification and, furthermore, that any actinomycete showing this character should be assigned to the genus *Nocardia*.

Generic classification. Inasmuch as the organism, *K 1,* exhibited the following characteristics, it was assigned to the genus *Nocardia:*

1. It produced a fairly compact colony composed of tangled mycelium and exhibited radiating, clubbed, branched, and curled elements at the periphery.
2. Fragmentation of the mycelium and post-fission movement (zig-zag arrangement) occurred at the periphery of the colony.
3. Arthrospores were produced.
4. Stained preparations revealed partial fragmentation into bacillary and coccoid forms.
5. Mycelial forms and spores averaged slightly less than 1 μ in diameter.
6. Neither nuclei nor septa were observed.
7. Branching was of an alternate type.
8. It was Gram-positive.
9. It was partially acid-fast.

Species identification. Study of the organism, *K 1,* has shown that it cannot be identified with any of the 33 species of *Nocardia* classified in Bergey's Manual.

Considering group I of the key, which contains 18 species, 4 of

these may be eliminated because they are not acid-fast. These 4 species, with additional differing characteristics, are as follows: *N. corallina* produces a pink pigment on agar; it produces a filiform smooth growth on an agar slant; it grows on glycerine potato; its optimum temperature is 22–25° C. *N. globerula* forms a watery colony 3–5 mm. in diameter on agar; it produces a filiform growth on an agar slant; it grows on glycerine potato and it has an optimum temperature of 25–27° C. *N. opaca* produces a cream-colored pigment on nutrient agar, produces a dry growth on glycerine potato and has an optimum temperature of 30° C. *N. erythropolis* forms a watery colony on agar about 2–3 mm. in diameter and produces a filiform growth on an agar slant; on potato it produces a dry, rough, orange-colored growth, and it has an optimum temperature of 25° C.

Of the remaining 14 species of group I, 6 form aerial mycelia. These 6 species, with additional differing characteristics, are as follows: *N. leishmania* produces a pink pigment and grows on carrot. *N. caprae* grows abundantly on potato and forms a red surface growth on milk. *N. pretoriana* forms a yellow to pink pigment and grows on potato. *N. pulmonalis* forms an abundant growth on potato, is strictly aerobic and liquefies gelatin. *N. paraffinae* is richly branching, produces a yellow pigment, forms hard smeary-surfaced colonies on agar and grows on potato. *N. transvalensis* forms a pink pigment; it grows on potato; it produces no change in milk.

Of the remaining 8 species of group I, 3 grow best at room temperature. These 3 species, with additional differing characteristics, are as follows: *N. polychromogenes* is richly branching; it is aerobic and it forms a pigment. *N. minima* forms a pink pigment; it grows on potato; it utilizes paraffin. *N. coliaca* produces a filiform growth on agar slant; it produces no coagulation in milk; it produces a dry orange to brown growth on glycerine potato; it produces no acid from glucose. *N. rubropertincta* grows well between 20 and 37° C.; it produces a red pigment; it grows on potato; it utilizes paraffin.

Of the remaining 4 species of group I, *N. asteroides* is strictly aerobic, grows well on practically any medium, produces large col-

onies, produces a pigment and is richly branching. *N. salmonicolor* produces a salmon to red color, grows on potato, utilizes paraffin and is a strict aerobe. *N. rubra* produces a red pigment, is aerobic and produces no change in milk. *N. farcinica* produces a yellow pigment, grows well on potato with a crumpled, abundant growth, does not change litmus milk, is markedly acid-fast and is only 0.25 μ in diameter.

Group II A, composed of *N. mesenterica, N. flava, N. viridis, N. citrea, N. madurae, N. blackwellii, N. cuniculi, N. rangoonensis,* and *N. caviae,* may be eliminated because they are not acid-fast. *N. mesenterica* is strictly aerobic, is richly branching, grows well on Sabouraud with hard, folded colonies, grows on potato, and induces no change in milk; *N. flava* forms a yellow pigment on meat peptone agar and produces shiny or rough and folded colonies; it produces no change in milk. *N. viridis* produces a green pigment; it produces no change in milk; it grows on potato; it does not produce any spores. *N. citrea* produces a yellowish-green pigment; it forms rough and folded colonies. *N. madurae* is a hardy aerobe; it grows on Sabouraud and on potato as well as in synthetic media. *N. lutea* grows well on agar with an abundant membranous pinkish colony; it grows on potato, and on glycerine potato with a carrot-red granular growth. *N. blackwellii* forms a yellow pigment, and forms a confluent wrinkled growth on agar; it grows on carrot, forming aerial spikes; it forms a yellow surface growth on milk. *N. cuniculi* grows on agar with a cream-colored, wrinkled growth, and on potato with a pink dry granular growth. *N. rangoonensis* branches profusely, forms aerial mycelia, and grows on carrot. *N. caviae* forms aerial mycelia and grows on potato.

Group II B, composed of *N. actinomorpha, N. flavescens, N. maculata, N. rhodnii,* and *N. gardneri,* are readily eliminated because they are not acid-fast; they are proteolytic; they form a pigment, and with the possible exception (characteristic not listed) of *N. maculata,* they grow on potato.

The following key, employing fundamental differences, shows the relationship of the organism, *K 1,* to the species of *Nocardia* classified in Bergey's Manual.

I. Acid-fast (14 species)
  A. Aerial mycelia produced (6 species)
  B. Aerial mycelia not produced (8 species)
    1. Optimum temperature 22–25° C. (3 species)
    2. Optimum temperature not listed (3 species)
      a. Produces a pigment, utilizes paraffin, grows on potato, is aerobic
        N. salmonicolor
      b. Produces a pigment, utilizes paraffin.....*N. rubra*
        N. rubropertincta
    3. Optimum temperature 37° C. (2 species)
      a. Aerobic (1 species)
      b. Facultative (1 species)
        Produces a pigment, grows well on potato, no change in litmus milk, markedly acid-fast, diameter of cell 0.25 microns
          N. farcinica
        Produces no pigment, no growth on potato, coagulates litmus milk, is partially acid-fast, diameter of cell 0.8–1.0 micron..*K 1*
II. Not acid-fast (19 species)

The following table shows a comparison of the organism, *K 1*, with the 14 species of *Nocardia* which are acid-fast (Bergey's Manual).

	aerobic	pigment production	growth on potato	aerial mycelia	growth (op) room temp.
K 1	−	−	−	−	−
N. leishmania	+	+		+	
N. caprae	+	+	+	+	
N. pretoriana	+	+	+	+	
N. pulmonalis	+		+	+	
N. paraffinae	+	+	+	+	
N. transvalensis	+	+	+	+	
N. polychromogenes	+	+		−	+
N. minima	+	+	+	−	+
N. coliaca	+	+		−	+
N. rubropertincta	−	+	+	−	
N. asteroides	+	+	+	−	−
N. salmonicolor	+	+	+	−	
N. rubra	+	+		−	
N. farcinica	−	+	+	−	−

Conant (**1948**) recognizes at least three species of *Nocardia* not among the 33 species classified in Bergey's Manual. These species and differing characteristics are as follows: *N. brasiliensis* (this is listed in the Bergey Manual appendix with the statement that according to Pinoy and others it is identical with *N. asteroides*) is a hardy, aerobic organism; on Sabouraud it produces yellow or orange dry, folded growth. *N. pelletieri* (described in Bergey's Manual as *Streptomyces pelletieri*) is strictly aerobic; it produces a pink to red pigment; it is not acid-fast; there is no fragmentation of mycelium; it produces aerial mycelia; it grows on potato. *N. paraguayensis* (listed in the Bergey Manual appendix) is not acid-fast; it produces on Sabouraud glucose agar a glabrous, soft, white-centered colony with darker adherent border; it does not fragment.

Cuttino and McCabe (**1949**) describe a new species, *N. intracellularis*, the etiologic agent in a fatal case of pure granulomatous nocardiosis. This organism produces a yellow pigment, grows well on Sabouraud medium, is aerobic, thrives at room temperature, and produces a yeast-like odor on all media. It is able to utilize paraffin.

In general, it may be said that the organism, *K 1*, differs sharply from previously described species of *Nocardia* in the following ways: (1) it is very fastidious in its growth requirements. It does not grow in synthetic media to which carbohydrates have been added; it will not utilize paraffin; it will not grow on potato or carrot; it will not grow on Sabouraud maltose agar nor on Sabouraud dextrose agar; attempts to grow it on nutrient agar and on BHI agar have given variable results; its optimum temperature is 37° C. (2) It is delicate and is relatively slow growing; it is never hardy or richly branching and it does not produce a surface scum or a confluent or filiform growth. (3) It is a facultative anaerobe, differing in this respect from all other *Nocardias* described with the exception of *N. farcinica* and *N. rubropertincta*, which are facultative.

The organism, here designated as *K 1*, is assigned to the genus *Nocardia*. Inasmuch as it is different from any other species of

Nocardia previously described, and shows a striking fastidiousness in its growth requirements, the specific epithet *fastidiosa* is selected.

Nocardia fastidiosa sp. nov.

Compages pleoformis, exigue ramosa, nodosa, fragilis; diametro mediocri mycelii 0.8 ad 1 μ; proferens arthrosporas; Gram-positiva; partim acido stabilis; aucta aut in aut sine aëre; lente crescens; post septem dies proferens in semisolida media discretas colonias 1 mm. diametro; in solida media albas, siccas, adherentes colonias 1 mm. diametro; delicatas et fastidiosas; augens optime aut in sanguine aut in medio cerebri-cordis; augens nec in Sabouraudi nec in ficto medio carbohydratas continente; optima temperatione 37° C.; non putrefacta; non manifesto perniciosa caviis, muribus, cuniculis.

Fons: ulcus in pene.

SUMMARY

An actinomycete was isolated in large numbers from a penile ulcer.

The actinomycete isolated was assigned to the genus *Nocardia* since it produces a ray-type mycelial colony with clubs, curls and branches, it fragments into coccoid and bacillary forms, it is Gram-positive and partially acid-fast.

This organism differs from previously described species of *Nocardia*. Since it shows a striking fastidiousness in its growth requirements, the designation *Nocardia fastidiosa* n. sp. is recommended.

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TAXONOMIC NOTES ON LOUISIANA FUNGI. III. ADDITIONS TO THE TREMELLALES

LINDSAY S. OLIVE

(WITH 51 FIGURES)

Fifteen species of jelly fungi are added to the list of twenty-seven already reported in the foregoing paper (**1948**) in this series. Most of these represent new records for Louisiana. Several new species are described.

MYCOGLOEA CARNOSA Olive. Mycologia **42**: 385. 1950.

This unusual auriculariaceous fungus was recently described as the type species of a new genus, for which no other species are known at the present time. A second and much earlier collection of the organism was recently observed by the writer. This specimen was found in material sent on loan from Kew Herbarium, and it had been included, without a name of any kind, on the same herbarium sheet with material labeled *"Tremella viscosa."* It came from the herbarium of Berkeley and had been collected on the bark of *Acer rubrum* in South Carolina by H. W. Ravenel.

Microscopically the Ravenel collection appears to be identical with the Louisiana fungus. The fructifications, however, are more extensive and are composed mostly of coalesced pustules which form a flattened and irregular, spreading mass.

Helicogloea parasitica sp. nov.

H. sebacinoidea Olive quoad basidia sporasque, stromate pulviniforme excluso. In *Exidia glandulosa* parasitans.

H. sebacinoidea Olive (**1948**) was described as having a thick, gelatinous fructification. A later collection discloses that what was originally supposed to be the fructification of the *Helicogloea* was in reality that of *Exidia glandulosa* parasitized by the *Helicogloea*. Since the original description was based on two distinct taxa, erroneously supposed to represent one, the name *Helicogloea seba-*

cinoidea is to be discarded as a *nomen confusum*, to be replaced by a more appropriate name referring to the *Helicogloea* alone.

At the time that the original description was made, no basidia of the host had been observed. However, a collection obtained later revealed the presence of both basidia and basidiospores of *Exidia*. The parasite is apparently capable of inhibiting sporulation in the host, so that the basidia and spores are either reduced in number or they fail to appear altogether.

A revised description of the fungus, including a correction of a previous error in recording spore dimensions, is given as follows:

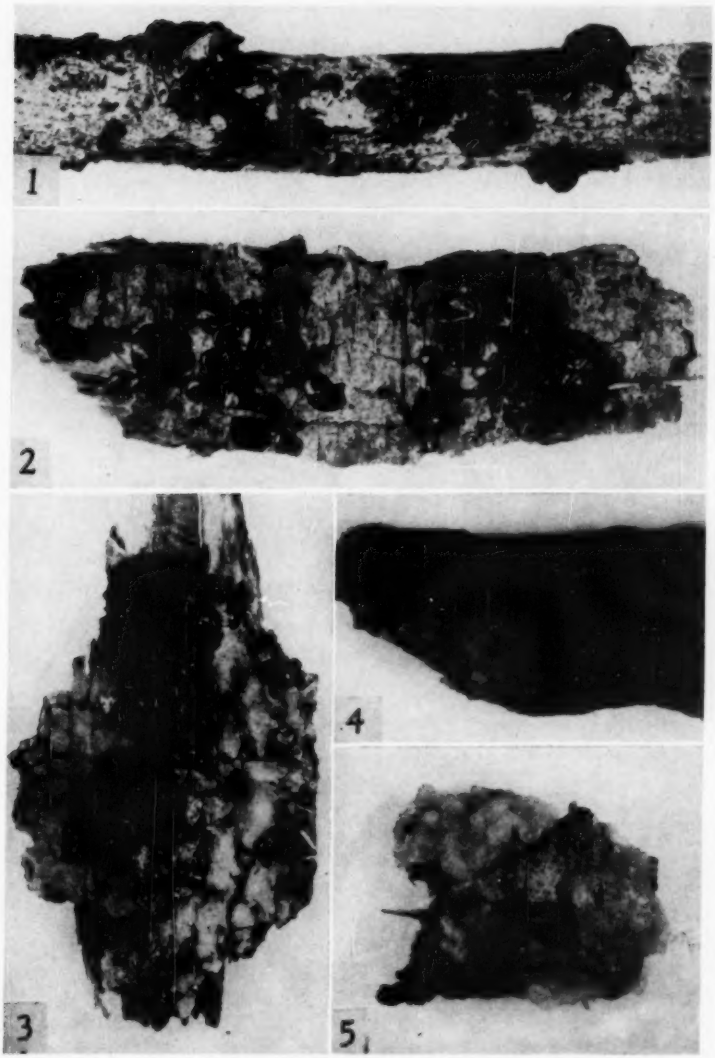
Parasitic within the fructifications of *Exidia glandulosa*. Vegetative hyphae within the fructification of the host, measuring 3.8–12.2 μ in diameter, without clamp connections; saccate, usually curved probasidia produced externally to the surface of the host fructification, measuring 10–14 × 25–50 μ, producing 4-celled basidia, 6.3–7.8 × 70–122 μ; basidiospores cylindrical, apiculate, straight or curved, 5.2–8.7 × 16.5–32 μ, germinating by repetition or becoming 1-septate and producing germ tubes.

Collected at Shreveport, July 4, 1947 (*La 110*, type) and at Baton Rouge, November 27, 1948 (*La 130*).

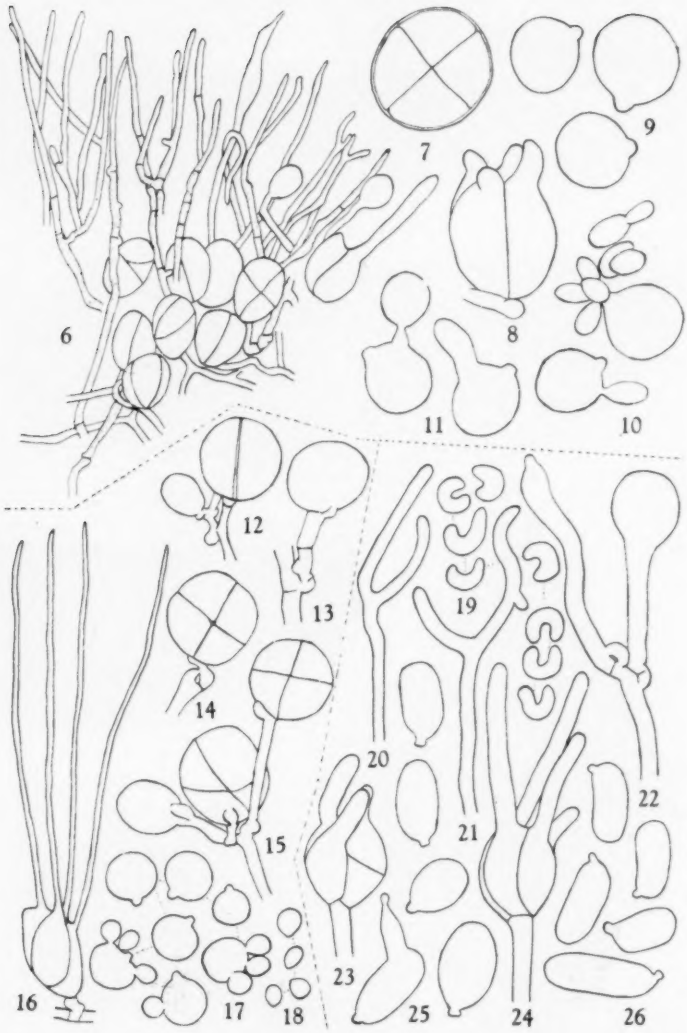
Tremella coalescens sp. nov. (FIGS. 3, 6–11)

Fructificationes plerumque sessiles, firme gelatinosae, atrobrunneae, nonnumquam cinnamomeo-brunneae, minores pulvinatae vel discoideae, in plagulas 2–3 cm. longit., ad 4 mm. crassit., denique gyroso-plicatas, coalescentes, exsiccatae crusta contracta atra factae. Hymenium e basidiis et paraphysibus brunneis constitutum; hyphae inconspicue nodoso-septatae. Basidia e globoso ovata, cruciatim septata, 19.1–26.1 × 24.4–33 μ; basisiosporae globosae, subglobosae, vel ample ovatae, apiculatae, 11.8–16.5 × 13–18.3 μ, frequenter conidia ovalia vel elliptica 3.8–5.7 × 5.3–9.1 μ gignentia. In ramo emortuo corticato quercino.

Fructifications sessile or with short tapered base, firmly gelatinous, blackish brown, occasionally varying to cinnamon-brown, smaller ones pulvinate or discoid, coalescing into conspicuous, somewhat gyrose-folded patches 2–3 cm. in length and up to 4 mm. in thickness; drying to a shrunken black layer. Hymenium composed of basidia and branched, septate paraphyses, the latter typically brownish with hyaline apical portions; brownish hyphae abundant deeper within the fructification; clamp connections present, but inconspicuous. Basidia subglobose to ovate, cruciately septate,



FIGS. 1–5. Photographs of Tremellales. 1. *Tremella moriformis* (× 4/3); 2. *Exidia repanda* (× 2); 3. *Tremella coalescens* (× 4/3); 4, 5. *Exidia tremelloides* (FIG. 4, × 1½; 5, nat. size).



FIGS. 6–11. *Tremella coalescens.* 6. Section of hymenium showing basidia and paraphyses; 7. Basidium in apical view; 8. Basidium in side view; 9. Basidiospores; 10. Basidiospores producing conidia; 11. Basidiospores, one germinating by repetition and one with germ tube. FIGS. 12–18. *Tremella moriformis.* 12–16. Basidia and probasidia; 17. Basidiospores, some of which are producing conidia; 18. Conidia. FIGS. 19–26. *Exidia tremelloides.* 19.

often with eccentric septa, 4-celled or occasionally 2-celled, generally tinted a light tan, measuring 19.1–26.1 × 24.4–33 μ, producing long sterigmata; deep-seated probasidia frequently enlarging into nearly empty brownish vesicles or occasionally developing into septate basidia which do not sporulate. Basidiospores globose, subglobose, or broadly ovate, occasionally as wide as long, distinctly apiculate, 11.8–16.5 × 13–18.3 μ, germinating by repetition or with germ tube, or budding out oval to elliptic conidia, 3.8–5.7 × 5.3–9.1 μ.

Growing on corticate oak limb, near Baton Rouge, November 27, 1948 (*La 129*, type).

The combination of certain characters such as the *Exidia*-like fructifications, firm texture, and brownish paraphyses distinguishes this fungus from all other species of *Tremella* known to the writer. It does not appear to be common, having been collected only once.

Tremella moriformis Berk. Outl. Brit. Fungol., p. 287. 1860. (FIGS. 1, 12–18)

T. moriformis has been reported in Britain, Europe, Ceylon, and in this country only from South Carolina. Coker (**1920**) examined a collection by Ravenel on deposit in the Curtis Herbarium. Several of Ravenel's South Carolina collections which are deposited at the New York Botanical Garden have been examined by the writer, and these proved to be identical with the Louisiana collection. Since the species is not well known in this country, a brief description of our collection is presented here.

Fructifications erumpent, moriform, 2–18 mm. in diameter, 1–8 mm. thick, dark purplish-brown to purplish-black, duplex, externally firmly gelatinous, internally somewhat spongy, with purplish-red core of densely interwoven hyphae, frequently discoloring the substrate with a purple or rose pigment. Hyphae with clamp connections, smooth-walled and hyaline at first, becoming roughened and purplish-brown with age. Basidia at various levels, often far beneath the surface, globose to oval or oblong, 4-celled, septa vertical to oblique or transverse, at first hyaline, becoming tinged a

Conidia from conidial fructification; 20, 21. Paraphyses; 22–24. Probasidia and basidia; 25. Basidiospores of various types from a single collection; 26. Basidiospores from another collection. (FIG. 6, × 395; 7–18, × 920; 19, × 1020; 20–26, × 1410.)

purplish-brown at maturity, measuring 14–19 × 18.3–27.8 μ, sterigmata often much elongated; basidiospores globose to subglobose, conspicuously apiculate, frequently depressed on one side, occasionally as broad as long, 7–10.5 × 8.7–13.6 μ, spore print white; basidiospores budding out small, globose to obovate or pyriform conidia, 2.3–6.1 × 3–8.4 μ.

Collected on corticate frondose limbs, south of Baton Rouge, February 3, 1949.

In the single locality where the fungus was collected, it occurred in great abundance. The dark tough fructifications, which resemble somewhat the fruits of mulberry and which discolor the limbs on which they are growing, readily distinguish this species of *Tremella* in the field.

Exidia tremelloides sp. nov. (FIGS. 4, 5, 19–26)

Fructificationes tenaci-gelatinosae, albae, parvae, 0.5–4.5 mm. diam., vel in massulas applanatas contortas 1–2.5 cm. longit., ad 3 mm. crassit., coalescentes. Basidia subglobosa vel ample ovata, in cellulis 4 divisa, 9.1–11.4 × 11.4–13.7 μ; basidiosporae e ovata cylindraceae, nonnumquam curvulae, 3.8–6.8 × 8.4–14.8 μ. Fructificatio conidiophora parva, conidia conspicue arcuatae 2.5–3.5 × 7–12.5 μ gignens. In ramis arborum frondosarum.

Fructifications white or whitish-hyaline, occasionally with sordid brown tints in some areas, varying in size from small hemispherical masses 0.5–4.5 mm. in diameter to flattened contorted masses 1–2.5 cm. broad by coalescence, up to 3 mm. thick, tough gelatinous; drying to a very inconspicuous to clearly visible vernicose film. Paraphyses conspicuous, sparingly branched; clamp connections present on the hyphae; basidia with basal septum forming late or sometimes not at all and with clamp connections considerably beneath the base, subglobose to broadly ovate, cruciately septate with septa vertical or oblique, measuring 9.1–11.4 × 11.4–13.7 μ; basidiospores ovate to cylindric, occasionally slightly curved, apiculate, 3.8–6.8 × 8.4–14.8 μ. Small conidial fructifications occasionally observed, conidia conspicuously curved, rarely straight, 2.5–3.5 × 7–12.5 μ, frequently found in small numbers in the basidial fructifications.

Eight collections on bark, rarely on decorticate portions, of frondose limbs, including oak, magnolia, and buckthorn; Baton Rouge, Avery Island, and Lake Verret (*La 139*, type). Common during March and April.

From the standpoint of the occasional presence of conidial fructifications, one might at first be inclined to place the fungus in the genus *Ditangium*. However, basidia later appear in these same fructifications, which eventually cease producing conidia; whereas in *Ditangium* the basidia always appear in separate fructifications.

In some respects, the new species is intermediate between *Tremella* and *Exidia*. This is particularly true with regard to spore form. The spores are rarely more than slightly curved. In some collections they are predominantly elongate, while in other collections they range from elongate to ovate in the same specimen. Conidium production by the basidiospores has not been observed.

At first it was thought that this fungus might be *Tremella albida* Huds., which was described by Hudson in 1778. However, type material of Hudson's species does not exist, and the description of it is not adequate to distinguish it from several other species of *Tremella* and *Exidia*. As a result of this uncertainty, various authors have classified entirely different jelly fungi under that name or under what they have considered synonyms of it. During the course of attempting to identify the Louisiana collections, the writer has found that there is a great deal of confusion in the literature, as well as in herbaria, concerning the identity of *T. albida*. In the following discussion, an attempt is made to clarify the status of this species.

In the absence of any type material of Hudson's fungus, the rules of nomenclature require that Fries's concept of the species be accepted. Fries (**1882**) recognized two types or forms under the name of *Tremella albida* Huds. Type *a* he called the ascending or rotund type and included as synonyms *T. cerebrina alba* Bull. and *T. candida* Pers. Type *b* was called the effused or applanate type, as typified by *T. candida* var. *effusa* Alb. & Schw., for which there is apparently no type material extant. However, Fries's brief description is based on type *a*, which he says is near *T. mesenterica* (*T. lutescens* (Pers.) Fr.), but smaller. Bulliard's first treatment of *T. cerebrina alba* Bull. (**1788**) is in the form of a figure (apparently of *T. mesenterica*) with legend; later (**1809**) he lists *T. mesenterica* as a synonym. Again there seems to be no type material in existence. Persoon (**1801**) described *T. candida*

as being similar to *T. mesenterica* in form. The only material of *T. candida* Pers. which the writer has been able to locate is in the Persoon herbarium at the Rijksherbarium in Leiden. Although the specimen could not be sent abroad on loan, the writer was informed of the following note appended to it by Neuhoff: "Material unzureichend! Anscheinend Spur von *Bourdotia cinerella* Bourd. & Galz." In other words, Neuhoff considered the material inadequate for an accurate determination, but stated that it appeared to be *Bourdotia cinerella* (*Sebacina caesio-cinera* (Höhn. & Litsch.) Rogers). Judging from this, one might also guess that Persoon included more than one fungus under the name of *Tremella candida*. At any rate, it is certainly not what Hudson had, nor is it what Fries had in mind.

More recently Neuhoff (**1935**) has attempted to define *Tremella albida* which he recognizes as *Exidia albida* (Huds. ex Loud.) Bref. Among the synonyms are included *T. albida* (Huds.) Loudon, *E. albida* Bref., *E. thuretiana* (Lév.) Fr., and *T. viscosa* Berk. & Br. Thus the post-Friesian treatment of Loudon is accepted because Neuhoff believed that Fries had confused several species under the name of *T. albida* Huds. In 1888, Brefeld described an *Exidia* which he thought, apparently without valid basis, was synonymous with *T. albida* Huds. and called it *E. albida* nov. sp. This explains the origin of the name used by Neuhoff. However, in view of the fact that the rules of nomenclature require that the concept of Fries be accepted, Neuhoff's treatment of the species cannot be considered valid. Since there is no type material of *T. albida* Huds. in existence and since the fungi Fries treated under this name probably represent more than one species, but primarily *T. lutescens* (*T. mesenterica*), it is suggested that the name *T. albida* Huds. be considered invalid. In this case *Exidia thuretiana* (Lév.) Fr. would probably be the correct name for the species with which Neuhoff is concerned here.

The Louisiana collections differ from *E. thuretiana* in several respects, including spore size and shape, color and texture when moist, appearance when dry, and the tendency of some of the younger fructifications to produce curved conidia. In its microdimensions *E. candida* Lloyd somewhat resembles the new species. However, the former has a much more compact hymenium and a

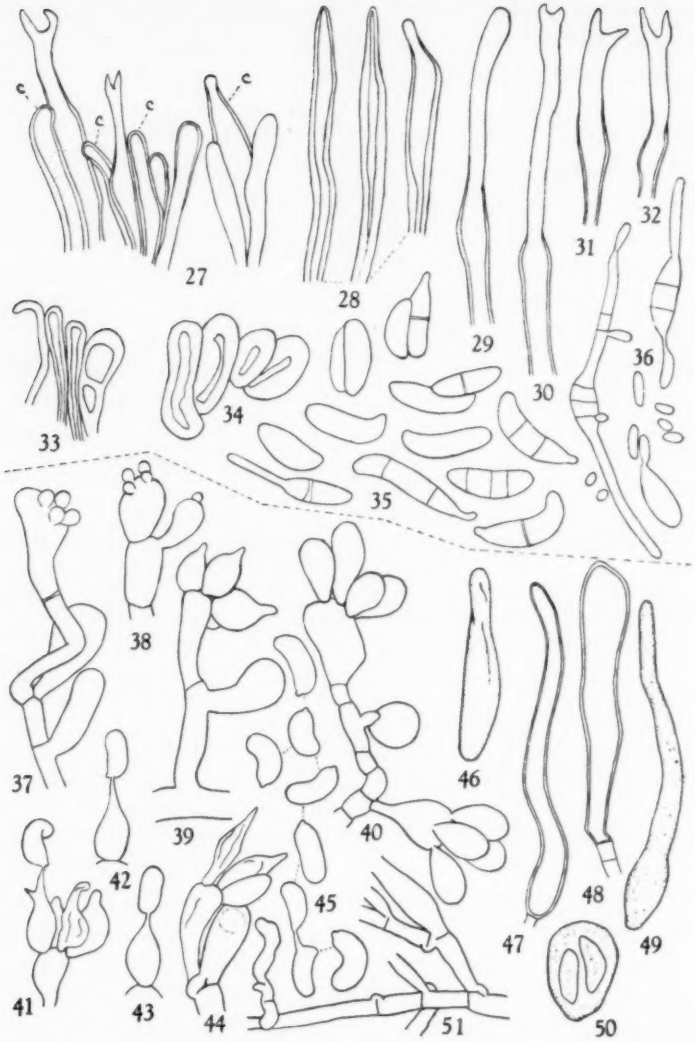
less gelatinous texture; also its basidia generally have clamp connections directly at their bases and the basidiospores are more curved and *Exidia*-like in appearance.

Exidia repanda Fr. Syst. Myc. **2**: 225. 1822. (FIG. 2)

Martin (**1944**), in his monograph of the Tremellales of the North Central United States and adjacent Canada, reports this species only from Ontario. At the New York Botanical Garden there are three collections from Alabama (one on pine), one from Maine, and one from New Jersey. If, as Neuhoff believes, *E. beardsleei* Coker is synonymous with *E. repanda*, then the range may be extended into North Carolina. The species is well known in middle and northern Europe, but, in spite of the fact that it seems to have a wide range in this country, it is seldom collected and not well known here. A description of the Louisiana collection is given below.

Fructifications firmly gelatinous, smoky brown to blackish brown, discoid, concave to convex, occasionally sterile, larger fructifications sometimes becoming gyrose folded, with or without short stalk-like base, 1–12 mm. in diameter, up to 8 mm. high, occurring singly or in clusters up to 5 cm. in length, frequently anastomosing, but with margins of individual fructifications remaining distinct, drying to shrunken black patches. Hyphae with clamp connections, hymenium containing brownish paraphyses which later gelatinize and leave the basidia embedded in a brown gelatinous layer. Basidia subglobose to ovoid or pyriform with septa frequently eccentric, 11.8–13.9 × 15.6–20.9 μ; basidiospores apiculate, conspicuously curved, 4.5–6.8 × 13.7–23.5(–26) μ, germinating by repetition. Growing on decorticate parts of frondose wood, probably oak, Baton Rouge, November 27, 1948.

The species is not common in Louisiana, only one collection of it having been obtained by the writer in that area. It differs from the description of Martin (**1944**) and Neuhoff (**1935**) mainly in its somewhat larger spore size and darker color. Martin lists the spore size as 3–4 × 12–13 μ and Neuhoff, 3–4(–6.5) × (10–)12–15(–17) μ. The writer has examined a specimen from the herbarium of Bresadola which has spores measuring 3.5–5.3 × 12.5–17.8 μ. Furthermore, the appearance of the spores and the structure of the hymenium (with basidia embedded in a brown gelat-



FIGS. 27–36. *Dicellomyces glocosporus.* 27. Portion of hymenium, showing probasidia, basidia, and gloeocystidia (c); 28. Gloeocystidia; 29–32. Basidia; 33. Sterile elements from margin of fructification; 34. Vesicular cells with thick gelatinous walls from outer part of fructification; 35. Basidiospores, some with septa and germ tubes; 36. Basidiospores producing germ tubes and conidia. FIGS. 37–51. *Glocotulasnella Rogersii.* 37–40. Probasidia

inous layer) strongly suggest that our fungus is the same species. Both Martin and Neuhoff note that the fructifications may become darker with age. None of the fructifications in the Louisiana collection showed the lighter brown colors which are said to be characteristic of younger fruiting bodies.

Dicellomyces gloeosporus Olive. Mycologia **37**: 543. 1945. (FIGS. 27–36)

This interesting leaf parasite of *Arundinaria*, which was described by the writer as the type for a new genus of the Dacrymycetaceae, has proved to be fairly common in Louisiana. Material collected near Baton Rouge showed heavy infection by the parasite. The original description of the fungus was based upon observations on dried, non-living material. Since the present observations are the first that have been made on living material and since these later studies have revealed certain important details not included in the original description, additional notes on the organism are included here.

The fresh fructifications are rather firmly gelatinous, whitish to cream color or grayish tan, pulvinate to discoid, and 0.3–1.5 mm. in diameter. On drying, they do not shrink to any great extent. The dried fructifications become yellowish to yellowish orange after the material has aged.

Inconspicuous clamp-like structures have been found at some of the septa on the hyphae. Towards the interior of the fructification the hyphae tend to gelatinize rather readily. The lower surface of the fructification is composed of a compact layer of sterile vesicular elements with thick gelatinized walls and narrow lumina, very much as in certain other Dacrymycetaceae, such as *Guepiniopsis*. At the margin of the fructification these sterile structures become more slender as they intermingle with the hymenial elements and may occasionally resemble paraphyses. However, they are not found in the remainder of the hymenium, and it appears that no true paraphyses are present. The hymenium is composed of pro-

and basidia, the latter producing epibasidia; 41–44. Production of basidiospores; 45. Basidiospores, one of which is germinating by repetition; 46–50. Gloeocystidia; 51. Branching hyphae with clamp connections. (FIGS. 27–45, 51, × 1410; 46–49, × 610; 48, 50, × 920.)

basidia, basidia and gloeocystidia. The gloeocystidia, which are pointed or blunt-tipped and cylindrical to subulate, have walls of varying degrees of thickness and light yellow non-granular contents. The light yellow, oily looking globules which are exuded onto the surface of the hymenium when a fresh mount is made apparently come from the gloeocystidia. Neither the sterile vesicles nor the gloeocystidia were reported in the original description. However, a re-examination of the type specimen reveals their presence there also.

The basidia, which arise apically from the probasidia, are 2-sterigmate and 9.1–34 μ in length. The basidiospores are elongate, curved to nearly straight and, although they frequently appear singly, they may also commonly be found adhering in pairs or in larger groups. They measure 2.3–4.1 × 7.6–14.4 μ and typically become 1–3-septate. Upon germination the spores may produce slender germ tubes at one or both ends, or they may bud out small oval to cylindric conidia. A single spore may germinate in both ways; also conidia may be budded out by the germ tubes.

The fungus was collected in great abundance on leaves of *Arundinaria tecta* near Hammond, March 21, 1947, and near Baton Rouge, April 11, 1949.

Recently, the writer examined a collection of diseased *Arundinaria* leaves from the herbarium of J. C. Arthur and now on deposit in the mycological collections at the New York Botanical Garden. The parasite proved to be *Dicellomyces gloeosporus*. It was collected on April 17, 1907, at Clemson, South Carolina. The fungus has now been reported from the following states: North Carolina, South Carolina, Georgia, Alabama, and Louisiana. It will probably be found eventually in all the Southeastern states.

Gloeotulasnella Rogersii sp. nov. (FIGS. 37–51)

Fructificatio tenuis, effusa, ceraceo-gelatinosa vel molliter gelatinosa, subhyalina, grisea, violaceo-grisea, vel alutacea, tuberculosa vel laevis, sicca crusta vernicosa vix visibilis. Hyphae plerumque rectae, 1.5–6.8 μ, nodoso-septate vel enodosae. Gloeocystidia abundantia, suco luteo viscoso suffulta, obovata vel clavata vel elongata atque supra attenuata, 7.6–13.8 × 16–92 μ. Probasidia obovata, clavata, vel capitato-clavata, 4.5–6.3 × 8.4–15.2 μ; epibasidia plerumque quattuor, ovalia vel pyriformia, 3.8–5.3 × 6.1–7.6 μ; basidiosporae typice allantoideae, apiculatae, 2.6–3.4 × 6.1–8.8 μ. In ligno arborum frondosarum crescens.

Fructification a thin, effuse, waxy-gelatinous to soft-gelatinous layer, nearly hyaline to gray, violaceous gray, or tan; surface tuberculate or even; drying to a thin almost invisible vernicose film. Hyphae mostly perpendicular to the substratum, much-branched, 1.5–6.8 μ in diameter, clamp connections present or absent at the septa. Gloeocystidia abundant, filled with golden yellow, viscous contents, disintegrating readily in the presence of abundant moisture, very variable in shape, ranging from obovate to elongate and tapering toward the apex, typically the latter, 7.6–13.8 × 16–92 μ. Probasidia arising near the surface of the fructification, occurring singly or in groups of 2 or 3, obovate, clavate or clavate-capitate, 4.5–6.3 × 8.4–15.2 μ, usually producing 4 epibasidia terminally or sometimes laterally near the apex; epibasidia mostly oval to pyriform, 3.8–5.3 × 6.1–7.6 μ; basidiospores typically allantoid, apiculate, 2.6–3.4 × 6.1–8.8 μ, germinating by repetition or producing germ tubes.

Collected on corticate and decorticate frondose wood, near Baton Rouge, November 27, 1947 (*La 119*, type), and January 10, 1949.

Although at least two species of *Tulasnella* have spores which are more or less allantoid, the new species of *Glocotulasnella* is distinct from all other members of that genus in its production of allantoid spores. The species is named in honor of Dr. D. P. Rogers, whose monograph of the Tulasnellaceae is well known.

Seven other species of jelly fungi, not previously reported by the writer and most of which probably represent new distributional records, have also been collected. These are listed as follows: *Sirobasidium sanguineum* Lagerh. & Pat., *Stypella minor* Möller, *Sebacina dubia* (Bourd. & Galz.) Bourd., *S. plumbescens* Burt, *S. deminuta* Bourd., *Dacrymyces abietinus* (Pers.) Schroet., and *Cerinomyces crustulinus* (Bourd. & Galz.) Martin.

The writer is grateful to Dr. D. P. Rogers for his preparation of the Latin descriptions and for valuable assistance with taxonomic problems, also to the New York Botanical Garden for making available its herbarium facilities. Type material of new species has been deposited in the mycological collections at the New York Botanical Garden.

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ENTOMOGENOUS SPECIES OF HIRSUTELLA, TILACHLIDIUM AND SYNNEMATIUM

E. B. MAINS

(WITH 20 FIGURES)

Patouillard (**1892**) described the genus *Hirsutella* in an article entitled "Une Clavariée entomogène." It was based on a species, *H. entomophila*, described from a specimen on a beetle collected by Lagerheim in Ecuador. Patouillard stated that the fungus has the appearance of an *Isaria*. He, however, placed the genus in the Basidiomycetes and included two other species not on insects, *Pterula setosa* Peck and *Typhula gracilis* Berk. & Desm. The basidia of *H. entomophila* are described as scattered on the clavula, ovoid with 1–2 subulata sterigmata. He states that the spores are hyaline, citriform, $8 \times 6\,\mu$ and pointed at the ends.

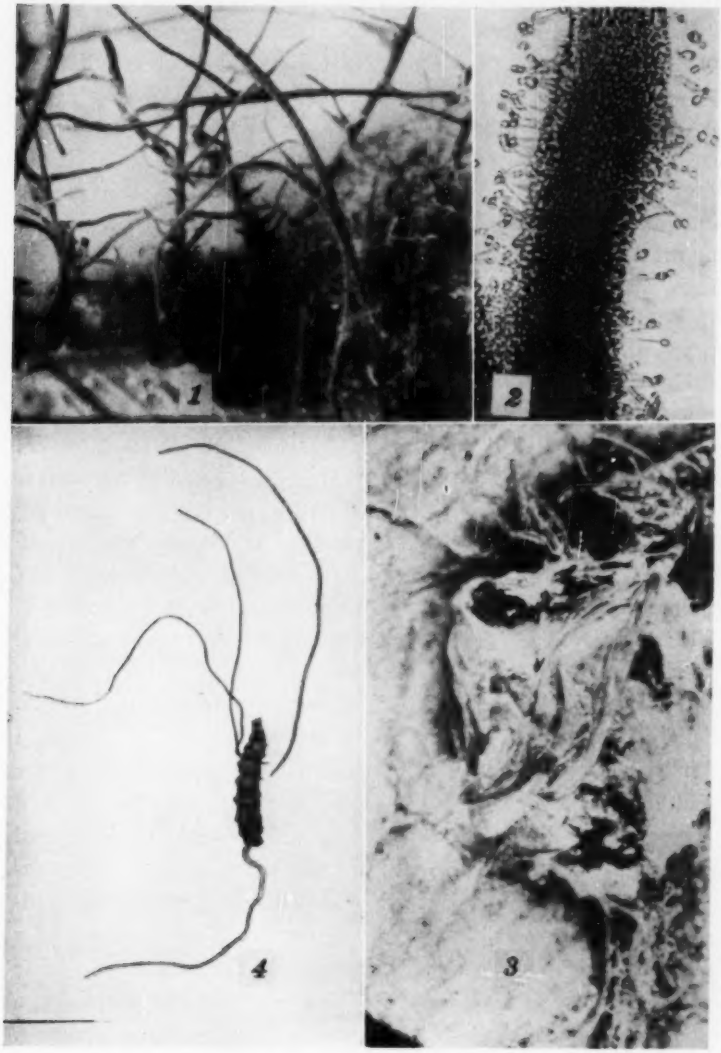
Speare (**1920**) concluded that *Hirsutella entomophila* is not a Basidiomycete but one of the Fungi Imperfecti. He points out that the conidia are produced singly at the tips of the sterigmata and are surrounded by a gelatinous substance, that the conidia of *H. entomophila* are fusiform, $7.5 \times 1.5\,\mu$ and that the description of Patouillard of citriform spores, $8 \times 6\,\mu$, apparently included the gelatinous covering. Speare included four additional species in the genus, *H. saussurei* (Cooke) Speare on *Polistes* spp., *H. floccosa* Speare on *Peregrinus maidis*, *H. citriformis* Speare on Homoptera and *H. fusiformis* Speare on a cricket. He published excellent illustrations of the species. Unfortunately most of the specimens studied by Speare have apparently been lost. They are not in the United States Bureau of Entomology where his studies were made or in the Mycological Collections of the United States Bureau of Plant Industry. A specimen of *Hirsutella citriformis* is in the Farlow Herbarium and it is proposed that it should be recognized as the type of that species.

Petch (**1923a**) proposed a genus *Trichosterigma* for similar fungi, including three species, *T. clavisporum* on a caterpillar, *T.*

arachnophilum on a spider and *T. attenuatum* on a Pentatomid. He included the genus in the *Stilbaceae*. Later (**1923b**) he decided that Speare's conclusions concerning *Hirsutella* were correct and that *Trichosterigma* was synonymous with that genus.

The type specimen of *Hirsutella entomophila* from the Patouillard collection in the Farlow Herbarium has been studied. It consists of a beetle larva covered with a yellowish mycelium (FIG. 3). The synnemata, which arise from all parts of the insect, are simple, slender, terete, slightly narrowing upward, 1–3 mm. long and 0.1–0.2 mm. thick. They are light brown and are finely setose from the phialides. They consist of longitudinal, slightly interwoven hyphae forming a compact bundle. The phialides are scattered to moderately crowded over the synnemata and arise mostly as lateral cells from the outer hyphae or less frequently as terminal cells at the ends of the hyphae. The lower portions of the phialides are narrowly ovoid or ellipsoid, 8–12 × 4–5 μ, abruptly narrowing at the apices into slender sterigmata, 12–20 μ long. Immature phialides without sterigmata occur intermixed with the mature phialides. The conidia are oblong, 5–8 × 1.5–3 μ. Most are covered with a persistent mucus forming a globose droplet 4–7 μ in diameter. Apparently, as Speare has suggested, Patouillard described the droplets when he states that the spores are citriform, 8 × 6 μ. Usually one conidium occurs in the droplet. Less often two are found. Some conidia without the mucus coverings were noted.

In the structure of synnemata and the origin and location of the philalides, *Hirsutella* resembles *Akanthomyces* and *Hymenostilbe* (see Mains **1950**) which have synnemata consisting of a compact bundle of longitudinal hyphae with the phialides occurring as lateral cells or on short lateral branches of the outer hyphae and forming a compact to loose hymenium over the surface of the synemma. This has been well illustrated by Speare for *Hirsutella* (**1920**, Pl. 3, Figs. 1 and 6). *Akanthomyces* and *Hymenostilbe* have phialides with sterigmata short or lacking and conidia with little or no mucus. In contrast, the species of *Hirsutella* have phialides with inflated lower portions and long slender sterigmata and conidia usually covered with a copious mucus (FIGS. 1–19). In species of *Hirsu-*

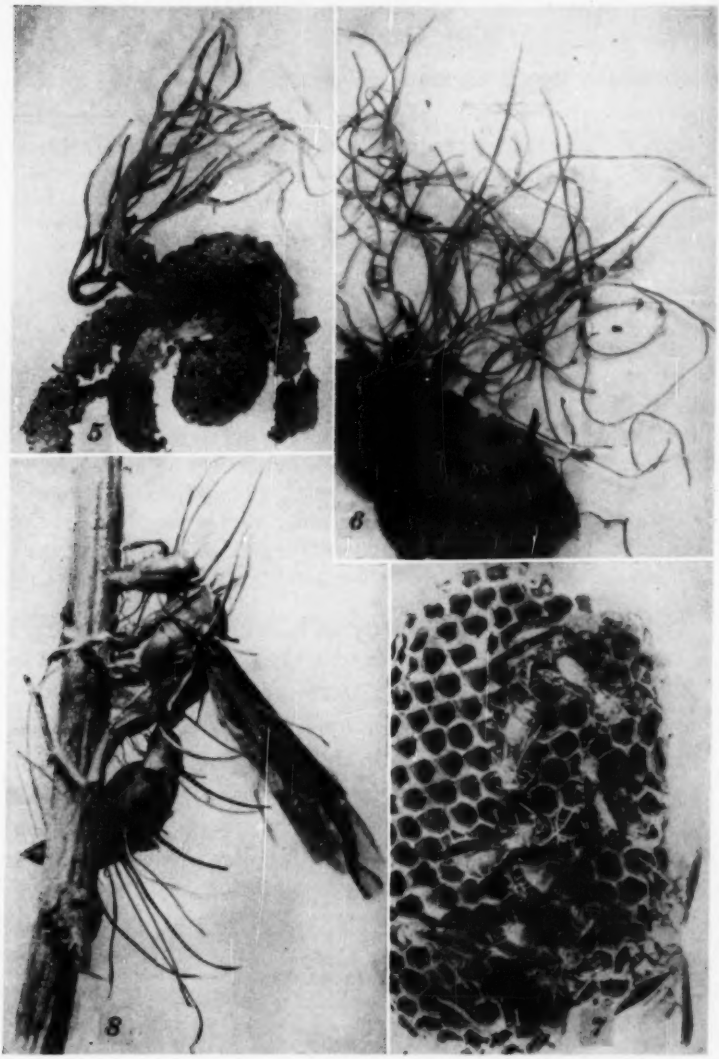


FIGS. 1–4. 1. *Hirsutella citriformis*, type, synnemata × 5. 2. *H. citriformis*, type, portion of synnema showing phialides and conidia, × 200. 3. *H. entomophila*, type, coleopterous larva and synnemata, × 8. 4. *H. barberi*, Van Dine, Puerto Rico, synnemata, × 2.

tella, only one conidium (FIG. 16) is usually found in the droplet of mucus at the apex of a sterigma. Occasionally two (FIG. 17) or rarely more occur, indicating that more than one conidium is produced by a phialide. Probably usually a conidium is detached before another is formed. When this does not happen the mucous coverings of the conidia coalesce, including them in an enlarged droplet of mucus. Speare has illustrated (**1920**, Pl. 3, Fig. 16) irregular clumps containing a number of conidia probably caused by the droplets of several phialides coalescing. In herbarium specimens the dried mucus is very persistent. Even after long standing in lacto-phenol preparations it remains undissolved and becomes practically an outer layer of the spore wall.

Entomogenous species having slender attenuated phialides and conidia covered with mucus have been reported in the genera *Tilachlidium* and *Synnematium.* The genus *Tilachlidium* differs from *Hirsutella* in several aspects. The type species is *T. pinnatum* Preuss (= *T. brachiatum* (Fr.) Petch) occurring on decaying fungi. This species has subulate phialides which mostly terminate the hyphae of the synnemata. Fewer are produced laterally. A number of conidia are produced from the apices of the phialides. These are irregularly arranged in spherical clumps similar to the condition found in *Cephalosporium.*

Synnematium was described by Speare (**1920**) based on *S. jonesii* Speare parasitizing *Mezira emarginata* and *M. lobata* in Louisiana. From the description and illustrations given by Speare and from a study of type specimens in the Farlow Herbarium *Synnematium* differs from *Hirsutella* and *Tilachlidium* in several important aspects. The conidia become brown in age. The phialides are not enlarged below as in *Hirsutella* but are subulate, gradually narrowing to the apex. They terminate the hyphae of the synnema and are not produced as lateral cells along the outer hyphae of the synnema as in *Hirsutella.* They bear globules containing a number of conidia similar to the condition found in *Cephalosporium.* A comparison of Speare's figures 1 and 4 of his Plate 4 with figures 1, 3, 4, 11, 12 and 14 of his Plate 3 shows these differences. *Synnematium jonesii* also produces sclerotia shown by Speare in figures 3, 5 and 6 of his Plate 4.



FIGS. 5–8. 5. *Hirsutella subulata*, Berryville, Va., Dickson (W) showing section through bark with larva below and synnemata above, × 2. 6. *H. gigantea*, FH *6199*, synnemata, × 8. 7. *H. saussurei*, on *Polistes rubiginosus*, Arkansas, Nest with infected wasps, × 0.75. 8. *H. saussurei*, on *Polistes annularis*, Georgia, synnemata, × 3.

This study is based mostly on North American collections in the Farlow Herbarium (FH),[1] the Herbarium of the New York Botanical Garden (NY), the Mycological Collections of the United States Bureau of Plant Industry (W), the Herbarium of the Florida Agricultural Experiment Station (Fl), and the Herbarium of the University of Michigan (M).

1. **HIRSUTELLA** Pat. Rev. Mycol. **14**: 69. 1892.

Synnemata cylindric to filiform, terete, usually somewhat attenuated upward, simple or branched, consisting of a compact bundle of more or less parallel, longitudinal, septate hyphae; phialides scattered to crowded over most of the synnema, mostly arising as lateral cells or buds or terminating short lateral branches along the outer hyphae of the synnema, a few developing as terminal cells of the hyphae of the synnema, occasionally developing on hyphae from the mycelial covering of the host, hyaline, inflated below, abruptly or gradually narrowing into long slender sterigmata; conidia oblong, subcylindric, fusoid to cymbiform, one-celled, hyaline, covered by a persistent mucus, single or 2–several occurring in droplets.

Type species: *Hirsutella entomophila* Pat.

A number of species of *Hirsutella* have been described which lack synnemata. The inclusion of these species in the genus is questionable. In the most generally accepted classification of the *Moniliales* such species belong in the *Moniliaceae* and those with synnemata, typified by *Hirsutella entomophila,* in the *Stilbaceae.* A classification which places little or no importance upon the union of conidiophores in synnemata necessitates an extensive revision of generic concepts and nomenclature in the *Moniliales.* This is not available. *Hirsutella,* therefore, is here treated as a stilbaceous condition of species whose final classifications and valid names will depend on their ascogenous conditions. It is probable that they will be species of *Cordyceps.* However, the ascogenous connections of most of the species of *Hirsutella* are not known.

[1] The writer is indebted to W. Lawrence White, Donald P. Rogers, John A. Stevenson and Erdman West, Curators in charge of the various herbaria, for the loan of specimens.

A. SPECIES ON ORTHOPTERA

HIRSUTELLA FUSIFORMIS Speare, Mycologia **12**: 70. 1920.

Synnemata simple, terete, slightly attenuated upward, 4–10 mm. long, 0.2 mm. thick, dark brown to nearly black; phialides arising as lateral cells or buds from the outer hyphae of the synnemata, the lower inflated portion fusoid, narrowing above into sterigmata 25–35 μ long; conidia fusoid, rounded at the ends, 9–10 × 2 μ, covered by a mucus.

On cricket, near Kingston, Jamaica, R. Thaxter (FH *6141*).

The species was described by Speare on a cricket from Hawaii. The specimen in the Farlow Herbarium from Jamaica was determined by Petch. Since an examination of this specimen resulted in a failure to find phialides and conidia the description has been partly adapted from the description and illustrations given by Speare (see his Pl 3, Figs. 6–9). An ascogenous connection is not known.

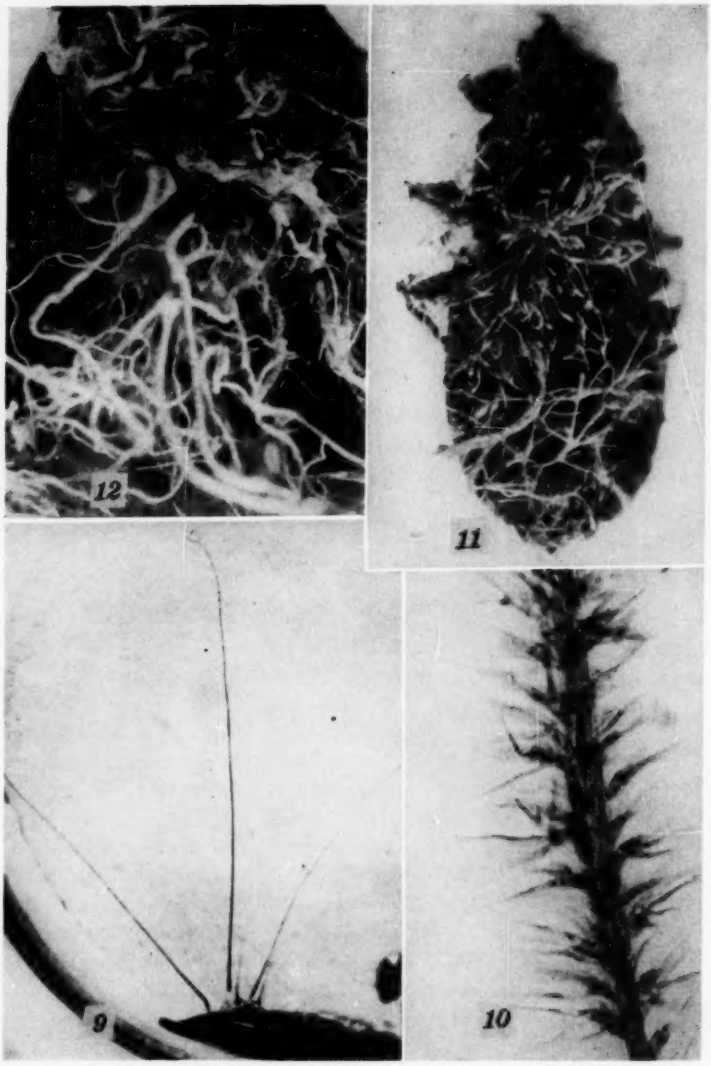
B. SPECIES ON HOMOPTERA

HIRSUTELLA CITRIFORMIS Speare, Mycologia **12**: 70. 1920. FIGS. 1, 2, 13.

Trichosterigma attenuata Petch, Trans. British Mycol. Soc. **8**: 215. 1923.

Mycelia brownish, more or less covering the hosts and attaching them to the substrata; synnemata aerial or procumbent, slender, up to 12 mm. long, terete, 0.2–0.3 mm. thick, sometimes simple, usually branched with short lateral branches, composed of a compact bundle of longitudinal hyphae, pubescent from the phialides; phialides forming a moderately compact layer over the surface of the synnema, mostly arising as lateral cells or less frequently as terminal cells of short lateral branches from the outer hyphae of the synnema, the inflated lower portions subgloboid, ellipsoid or subfusoid, 6–14 × 3–4 μ, narrowing at the apices into slender sterigmata, 30–40 μ long; conidia oblong, cymbiform to subfusoid, 5–8 × 2–2.5 μ, 1–2 in droplets of mucus 5–10 μ in diameter (FIG. 13).

Florida: on *Leptopharsa constricta,* Ft. Pierce, Sept. 10, 1945, N. Stahler (W); on *Corythuca ulmi,* Evinston, A. N. Tissot & J. O. Pepper (Fl *9345*).



FIGS. 9–12. 9. *Hirsutella radiata*, FH *6160*, synnemata. 10. *Thilachlidium ramosum*, type, portion of synnema showing phialides, × 400. 11. *Synnematium jonesii*, type, FH *4150*, synnemata, × 9. 12. *S. joncsii*, FH *5369*, dried agar culture showing synnemata, × 5.

Puerto Rico: on *Bothriocera venosa,* El Yunque Mts., Sept. 29, 1940, L. E. Gregory & L. F. Martorell (W); on *B. venosa,* Dec. 12, 1941, G. N. Wolcott (W).

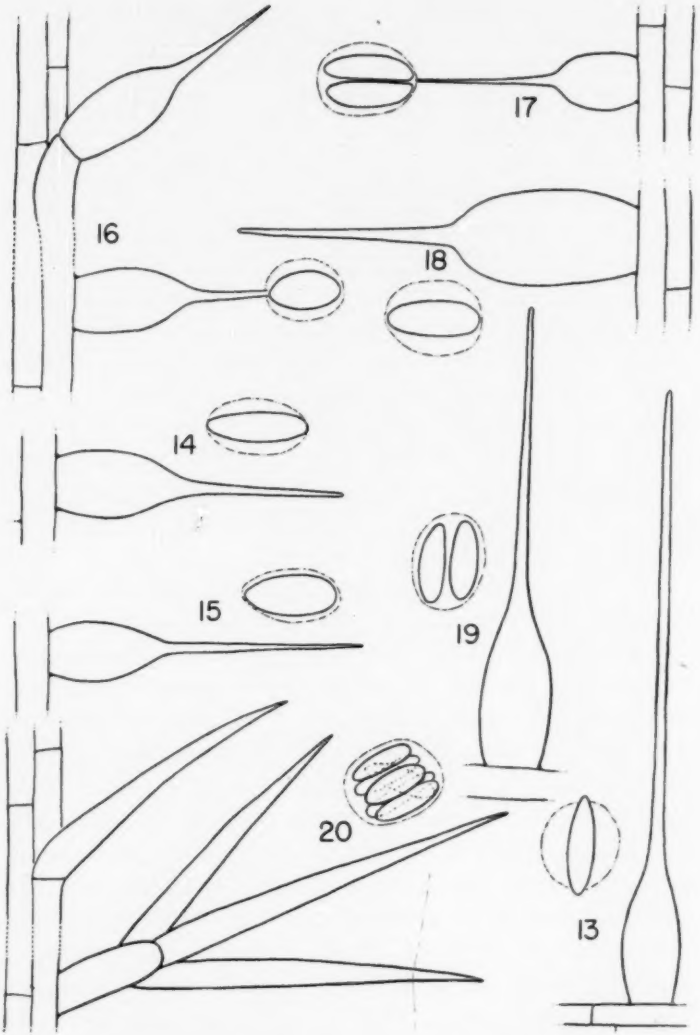
Hawaii: on *Syphanta acuta,* Oahu, A. T. Speare, type (FH *752*); on *S. acuta,* Oahu, A. T. Speare (M).

In establishing this species Speare (**1920**) cites, as hosts, Fulgoridae from New Zealand and Puerto Rico, *Ricania discalis* from New Zealand, *Perkiniella saccharicida* from Hawaii and *Siphanta acuta* from Hawaii. The specimen in the Herbarium of the University of Michigan on *Siphanta acuta* from Hawaii collected by Speare was in the duplicate collections of the Farlow Herbarium. It is similar to specimen *752* in the Farlow Herbarium, Harvard University (FIGS. 1, 2). Both were collected by Speare. Since specimens do not occur in the collections of the United States Department of Agriculture at Washington, specimen *752* in the Farlow Herbarium should be recognized as the type of *Hirsutella citriformis.* The specimen on *Siphanta* in the Herbarium of the University of Michigan is a paratype.

There is considerable variation in the size of the synnemata in this species. The collection on *Leptopharsa constricta* from Florida has synnemata 0.3–3.5 mm. long, mostly simple or with a few short branches. In contrast the collection on *Syphanta acuta* from Hawaii has synnemata up to 10 mm. long and much branched. These differences in development appear to be correlated with the size of the insect from which they arise.

The collection on *Bothriocera venosa* from the El Yunque Mts. of Puerto Rico differs from others in having many phialides produced at the ends of short lateral branches which arise from the outer hyphae of the synnemata. Speare (**1920**) has illustrated such branches in his Pl. 3, Fig. 15. He considers them to be abnormal developments. There is a tendency to produce such phialide-bearing branches throughout the species although sessile phialides are mostly produced.

Petch (**1923a**) described a species on a Pentatomid from Hakgala (India) as *Trichosterigma attenuatum.* He (**1923b**) later decided that it was the same as *Hirsutella citriformis.* It would appear therefore that the species also occurs on Hemiptera. An



FIGS. 13–20. 13. *Hirsutella citriformis*, phialide and conidium. 14. *H. entomophila*, phialide and conidium. 15. *H. stylophora*, phialide and conidium. 16. *H. barberi*, terminal and lateral phialides and conidium. 17. *H. subulata*, phialide and conidia. 18. *H. gigantea*, phialide and conidium. 19. *H. saussurei*, phialide and conidia. 20. *Tilachlidium ramosum*, single and grouped phialides and conidia. All × 2000.

association of *H. citriformis* with an ascogenous stage has not been observed.

Hirsutella abietina (v. Höh.) Petch (**1931**) has been reported on leaf hoppers from Java and India. According to the description by Petch it has phialides with ovoid bases 8–10 × 5 μ and sterigmata 16 μ long and ovoid or subcymbiform conidia, 5–6 × 2–2.5 μ which are aggregated into clusters of four spores. Petch (**1933**) has reported the conidial stage of *Cordyceps clavulata* (Schw.) Ellis & Ev. as *Hirsutella lecaniicola* (Jaap) Petch. For reasons published elsewhere the writer (**1950**) has placed this species in the genus *Hymenostilbe*.

C. SPECIES ON COLEOPTERA

HIRSUTELLA ENTOMOPHILA Pat. Rev. Mycol. **1892**: 69. FIGS. 3, 14.

Mycelium yellowish to brown, slight to dense, covering hosts and attaching them to the substrata; synnemata arising from various parts of the host, composed of parallel, longitudinal, closely compacted hyphae, very variable in length, 1–20 mm. long, terete, 0.1–0.2 mm. thick, simple or branched, often procumbent and attached to the substratum and producing short erect secondary branches, brown to light cream color in the younger portions, minutely setose; phialides scattered over the synnema, most arising as lateral cells from the outer hyphae of the synnema, the inflated lower portions ellipsoid to obovoid, 5–10 × 4–6 μ, abruptly narrowing above into slender sterigmata, 12–34 μ long; conidia fusoid to subcylindric, 5–8 × 1.5–2.5 μ, rounded at the ends, 1–2 included in droplets of mucus.

Trinidad: on beetles, Verdant Vale, R. Thaxter, 1912–13 (FH *2926, 2595, 2558*); Maraval Valley, R. Thaxter, March 8, 1913 (FH *2596*); Port-of-Spain, R. Thaxter, 1912–1913 (FH *622A*).

Jamaica: on *Diabrotica* sp. in airplane baggage at Miami, Florida, Milnor, March 7, 1938 (FI).

Ecuador: Pallatanga, Sept. 1891, de Lagerheim, type (FH).

The collections from Trinidad all developed on adult beetles. The synnemata of most of the specimens are considerably branched and appressed to the leaves on which the beetles were attached. The appressed condition was probably caused by the attachment of

the beetles to the undersides of the leaves. In these respects they differ from the type specimen which is on a coleopterous larva and has free simple synnemata. The collection from Jamaica was apparently found in an inspection of baggage at Miami, Florida, on an airplane from Jamaica. *Hirsutella entomophila* has not been found in association with an ascogenous stage.

Hirsutella eleutheratorum (Nees ex Fr.) Petch is a similar species on beetles in Europe and the two species may eventually be found to be the same. Petch (**1932b, 1939**) has suggested that *H. eleutheratorum* is the conidial stage of *Cordyceps entomorrhiza* (Fr.) Link. The connection is questionable (Mains **1951**).

Hirsutella stylophora sp. nov. FIG. 15.

Synnemata subulata, 1.5–4.5 cm. longa, 0.2–1.0 mm. crassa, obscure brunnea et pubescentia infra, griseo-brunnea et pulverulenta superne; phialides ellipsoideae, 6–10 × 3–4 μ, sterigmatibus 8–22 μ longis; conidia fusoidea vel obovoidea, 5–8 × 2.5–4 μ, muco tecta.

Ex larva scarabaei, Kent Lake, Mich. A. H. Smith, *7554*. Herb. Univ. Mich.

Synnemata arising directly from larvae or as branches of the perithecial clavae, subulate, 1.5–4.5 cm. long, 0.2–1.0 mm. thick, dark brown and pubescent below, grayish brown and slightly pulverulent above; phialides produced on the synnemata and terminal portions and branches of the perithecial clavae, scattered, arising from the outer hyphae of the synnemata, the inflated lower portions ellipsoid, 6–10 × 3–4 μ, narrowing above into slender sterigmata 8–22 μ long; conidia fusoid to obovoid, 5–8 × 2.5–4 μ, covered with a slight mucus.

Michigan: Kent Lake, Aug. 26, 1937, A. H. Smith, *7311* (M); Kent Lake, Oakland Co., Sept. 13, 1937, A. H. Smith, *7554*, type (M).

New York: Catlin Lake, Adirondack Mts., Aug. 19, 1934, A. H. Smith, *395* (M); Warrensburg, Sept. 12, 1934, A. H. Smith, *843* (M).

Tennessee: Great Smoky Mts., Nat. Park, Aug. 22, 1938, A. H. Smith, *10400* (M); Indian Creek, Great Smoky Mts. Nat. Park, Aug. 1, 1943, Arthur Stupka (M).

This is the conidial stage of *Cordyceps stylophora* Berk. & Br. The conidial stage has not been previously described. It is desir-

able to have a name for it since it sometimes occurs alone. Consequently it is described as a new form-species of *Hirsutella*.

The conidial development precedes that of the perithecia oftentimes upon the same fructification. The sporophores start their development in the summer and probably first produce conidia. In some instances perithecial stromata develop forming a cylindrical covering of the central third of the sporophore and the upper accumunate portion bears conidia. Sometimes conidia-bearing branches may be produced below the stromata. This species appears to be unique in that asci and ascospores mature after overwintering (Mains, 1941). This species of *Hirsutella* does not produce as conspicuous a covering of mucus over the conidia as in most of the species of the genus. The long sterigmata are characteristic of *Hirsutella*.

Petch (**1937**) has suggested that the conidial stage of *Cordyceps variabilis* on larvae of Coleoptera in North America is a *Hirsutella*.

D. SPECIES ON LEPIDOPTERA

HIRSUTELLA BARBERI (Giard) Petch, Trans. British Mycol. Soc. **21**: 58. 1937. FIGS. 4, 16.

Isaria barberi Giard, Compt. Rend. Soc. Biol. **46**: 824. 1894.

Larvae covered with a slight mycelium; synnemata arising from various parts of the host, slender, terete, slightly attenuated upward, up to 30 mm. long, 0.1–0.4 mm. thick, grayish brown, composed of closely compacted parallel longitudinal, 2–4 μ wide hyphae; phialides widely separated, arising as lateral cells or lateral buds or occasionally as terminal cells of the outer hyphae of the synnemata or from the mycelium, the lower portions short cylindric, obovoid or hemispheric, 4.5–10 × 3–4 μ, narrowed above into slender sterigmata 4–9 μ long; conidia ellipsoid to obovoid, 4–6 × 3–4.5 μ, contained in a small droplet of mucus or sometimes with slight or no evident covering of mucus.

Cuba: on larva of *Diatraea saccharalis*, April 22, 1929, H. K. Plank (W).

Puerto Rico: on larva of *Diatraea saccharalis*, March 26, 1913, J. A. Johnston, Lloyd Mycol. Herb. *37314* (W); on larvae of *Diatraea saccharalis*, Guanica, 1913, Van Dine (W); larva of *Diatraea saccharalis*, Guanica, Feb. 1940, L. F. Martorell (W).

Jamaica: on sugar cane borer, A. H. Ritchie, Lloyd Mycol. Herb. *42604* (W).

This species appears to be a fairly common parasite of the sugar-cane borer, *Diatraea saccharalis* in the West Indies. Miss Charles (**1941b**) has reported it on *D. lineolata*. It has also been reported for the Barbados, Trinidad, Dominican Republic, and Louisiana (Petch, **1937**; Charles, **1941a**).

Hirsutella barberi was described by Giard (**1894**) from specimens on *Diatraea saccharalis* collected by C. A. Barber in St. Christopher and the Barbados of the West Indies. He also states that the fungus had been seen on Antigua. Massee (**1895**) described a fungus in the ascogenous stage which he states occurred on specimens at Kew sent by John R. Bovell labelled *Cordyceps bovellii*. On the assumption that *Isaria barberi* was the conidial stage he employed the name *Cordyceps barberi*. Lloyd (**1916**) and Petch (**1937**) state that they were unable to find collections in the Kew Herbarium to which Massee's description applies. The application of the name *Cordyceps barberi* is therefore doubtful.

HIRSUTELLA SUBULATA Petch, The Naturalist **1932**: 48. FIGS. 5, 17.

Larvae covered with a slight mycelium; synnemata caespitose, slender, terete, slightly attenuated upward (5)–15–50 mm. long, 0.1–0.3 mm. thick, light brown to gray, simple or furcate, composed of closely bound, parallel, longitudinal hyphae 2.5–3.0 μ wide; phialides scattered, arising as lateral cells or buds of the cells of the outer hyphae of the synnemata or from the hyphae of the mycelium, the lower portions narrowly ellipsoid, broadly obovoid or conoid, 4–8(–10) × 3–5 μ, abruptly narrowing above into slender sterigmata 6–12 μ long; conidia narrowly ellipsoid, often slightly asymmetric, more convex on one side than the opposite, 4–8 × 1.5–2.5 μ, 1 or 2 in spherical droplets of mucus 6–7 μ in diameter.

Delaware: on larvae of *Carpocapsa pomonella*, F. S. Zimmerman, Nov. 1930 (W).

District of Columbia: on codling moth, Washington, Riley (FH *6241*).

Indiana: on *Carpocapsa pomonella*, Vincennes, 1927, Alvah Peterson (W).

Maine: on larvae of *Apatela dactylina,* Kittery Pt., Aug. 1891, R. Thaxter (M).

New Jersey: on codling moth, no location or date given, R. Thaxter? (M).

New York: on larva of *Aegeria pyri* in apple bark, Flatbush, Long Island, Sept. 23, 1890, J. L. Zabriskie (FH *6230*).

Ohio: on *Pyrausta nubilalis,* 1935, Armond (W).

Virginia: on codling moth larvae, Rosemont Orchard, Berryville, 8–6–40, Dickson (W); on *Carpocapsa pomonella,* 5 mi. N. W. Winchester, Sept. 9, 1940, W. S. Hough (W); on *Conopia* (*Synnanthedon*) *pyri* on *Pyrus malus,* Maychild Orchard, Mt. Jackson, May 18, 1941 (W); on *Carpocapsa pomonella,* Byrd Orchard, Berryville, 5–1–40, Hough (W); on *Carpocapsa pomonella,* Berryville, May 20, 1940, V. K. Charles (W).

Petch (**1932b**) described the species from a specimen in the Kew Herbarium on a caterpillar from England which had been reported by Berkeley and Broome (**1881**) as *Isaria floccosa* Fr. Petch has questioned the determination and has also pointed out that the combination *Hirsutella floccosa* has been employed by Speare for another fungus. Petch described the conidia as cymbiform, 2.5 × 1 μ. In a later publication he (**1937**) discussed two collections from the Farlow Herbarium, nos. *6230* and *6241,* and gave the measurements of the conidia as 4–6 × 1.5–2 μ and 4 × 1.5 μ. Miss Wakefield has kindly had the type specimens in the Kew Herbarium examined and states that as far as can be determined from the few conidia seen they measure 5–6 × 1.5–2 μ. The measurements given by Petch in his original description apparently are erroneous.

In the United States this species sometimes becomes an important parasite of codling moth, *Carpocapsa pomonella.* Miss Charles (**1941b**) has reported that as high as 40% of the larvae of codling moths were killed in an apple orchard at Berryville, Virginia, in 1940. She found that the fungus grew well on corn meal agar and Thaxter's potato-dextrose agar but did not produce synnemata. Synnemata were produced on infected larvae after being kept at a cool temperature for ten days.

The report of the species for the European corn borer, *Pyrausta nubilalis,* is based on a specimen determined as *Hirsutella* sp. in

the Mycological Collections of the Bureau of Plant Industry. It has short synnemata bearing the characteristic phialides with long sterigma and conidia in droplets of mucus of *H. subulata*. With the specimen there is a note stating "Larva showing fungus in incubator room 1920 Parkwood Ave., Toledo, Ohio. Larva collected in the vicinity of Toledo, Ohio, fall of 1935. Communicated by Dr. Armond."

Hirsutella subulata has not been found associated with an ascogenous stage. It is very similar to *H. barberi*. It has a different distribution and the conidia tend to be asymmetric. The relationship of the species probably cannot be determined until the ascogenous stages are known.

HIRSUTELLA GIGANTEA Petch, Trans. British Mycol. Soc. **21**: 48. 1937. FIGS. 6, 18.

Synnemata several, simple or branched from near the base, terete, slightly attenuated upward, 2–10 cm. long, 0.5–1.0 mm. thick, olivaceous below, brown above, pruinose to minutely setose, composed of closely compacted parallel longitudinal, 2.5–3 μ wide hyphae; phialides scattered to crowded, developing as lateral cells from the outer hyphae of the synnemata, the lower portions oblong to ellipsoid, 10–18 × 5–8 μ, abruptly narrowing into slender sterigmata 10–20 μ long; conidia oblong, slightly narrowing at each end, 5–8 × 2–2.5 μ, 1–2 or rarely more in droplets of mucus 6–16 μ in diameter.

Connecticut: New Hartford, 1888–1889, R. Thaxter (FH).

Maine: on cocoon of *Apatela*, York, R. Thaxter (FH *6199*).

New Hampshire: on pupa of *Apatela americana*, above Diana's Bath, Intervale, July 1901, R. Thaxter, type (FH *6126*).

A specimen of a *Hirsutella* on the spruce budworm, *Choristoneura fumiferana*, received from Donald M. MacLeod of the Forest Insect Laboratory, Sault Ste. Marie, Ontario may be this species. The synnemata are very short, not more than 5 mm. long. Their size may be due to immaturity and the small larvae on which they occur. The phialides and conidia agree with those of *H. gigantea*.

Petch (**1937**) has suggested that *H. gigantea* may be the conidial stage of *Cordyceps elongata* Petch. The stages have not been found associated. This species has larger, more cylindric phialides than

H. barberi and *H. subulata*. The synnemata according to the description given by Petch are much longer. This may be correlated with the larger larvae from which they developed. *Hirsutella exoleta* (Fr.) Petch on lepidopterous pupae from England has brownish black synnemata. The phialides and conidia do not differ greatly from those of *H. gigantea*. Petch (**1939**) describes the phialides as having inflated flask-shaped bases 12–18 × 5 μ and sterigmata up to 20 μ and cymbiform conidia 9 × 2.5 μ.

Hirsutella clavispora Petch (**1923b**, **1924**) was described on a caterpillar from Peradenija, Ceylon. The conidia are given as cylindric or clavate, 4–8 × 1–1.5 μ, with a mucus, and the sterigmata as very short, 5–9 μ. Petch (**1932a**) has suggested that this is the conidial stage of *Cordyceps tuberculata* (Lebert) Maire. The conidia of that species do not have a mucus and are in chains (Mains, **1950**). *Hirsutella ramosa* Mains (**1949**) has also been described on remains of a lepidopterous insect from Nova Scotia. A restudy of this collection has resulted in the conclusion that it is a *Tilachlidium*.

E. SPECIES ON DIPTERA

HIRSUTELLA RADIATA Petch, Trans. British Mycol. Soc. **19**: 184. 1934. FIG. 9.

Synnemata simple or branched, erect or appressed to the substrata, very slender, terete, 4–20 mm. long, 70 μ thick, dark brown, cinereous toward the tips; phialides conoid or cylindric below, 5–18 × 2–4 μ narrowing into slender sterigmata 6–14 μ long; conidia cymbiform to ovoid, 6–9 × 2–4 μ, agglutinated into ovoid spore-clusters 9–11 × 6–7 μ.

North Carolina: on Diptera, Cranberry, Aug. 1887, R. Thaxter (FH *6156, 6160*).

British Guiana: on flies, Essequibo, Sept. 10, 1916, C. B. Williams (FH *722*).

There are three specimens in the Farlow Herbarium determined by Petch. Specimen No. *722* is fragmentary. Petch based his description of the species on specimens collected by C. B. Williams on Sept. 11, 1916. Phialides and conidia were not found on the two specimens from North Carolina. The description above is

mostly adapted from that given by Petch. His figure shows a very slender synnema which has four long branches from its apex, one of which has secondary branches from its upper portion. Petch does not state that the conidia occur in a droplet of mucus but occurrence as a spore cluster indicates it. Specimen *6160* in the Farlow Herbarium has erect simple slender synnemata up to 20 mm. long. Specimen *6156* has slender appressed synnemata apparently due to the attachment of the host to the underside of a leaf. The ascogenous stage is unknown.

Hirsutella dipterigena Petch (**1937**) has been described on flies from England. According to the description, it has smaller phialides and longer sterigmata than *H. radiata*.

F. SPECIES ON HYMENOPTERA

HIRSUTELLA SAUSSUREI sensu Speare, Mycologia **12**: 69. 1920. FIGS. 7, 8, 19.

Isaria crinita Lloyd, Mycol. Writings **6**: 919. 1920.

Synnemata aerial or procumbent and appressed to the substratum, arising from various parts of the body and appendages of the host, slender, terete, somewhat attenuated upward, very variable in length, 1–17 mm. long, 0.1–0.3 mm. thick, light brown, composed of closely compacted, parallel, longitudinal, 2–4 μ wide hyphae; phialides scattered to moderately crowded, arising as lateral cells or buds from the outer hyphae of the synnema or as terminal cells of short lateral branches, the lower portions cylindric, obovoid or ellipsoid, 7–16 × 3–6 μ, attenuated above into slender sterigmata 10–40 μ long; conidia oblong to cymbiform, 5–8(–10) × 1.5–2.2 μ, 1 or more in droplets of mucus 5–10 μ in diameter.

Arkansas: on *Polistes rubiginosus*, 1950 from Sgt. James Gentry (M).

Florida: on *Vespula* sp. Ocala Nat'l Forest, Marion Co., 6–11–1938, T. H. Hubbell (M); on wasp, T. Van Hyning, Lloyd Mycol. Col. *42608* (W); on *Polistes annularis*, Homestead, 1–15–26, Mrs. C. H. Eigenman (loaned by J. Bequaert, Mus. Comp. Zoo. Harvard).

Georgia: on *Polistes annularis*, Savannah, June 6, 1949, Joe Carter (U. S. Bur. Ent. Pl. Quar. Lot 49–8624).

North Carolina: on Ichneumonfly, Cranberry, 1887, R. Thaxter (FH *6261B*).

Honduras: on wasp, Mt. Vyuca, Zamorave, alt. 5800 ft., July 12, 1948, T. H. Hubbell (M).

Panama: on wasps, Changuinola, June, 1921 (FH *6166* and NY).

Puerto Rico: on social wasp (Vespidae), San Sebastian farm, Nov. 14, 1935, M. R. Smith (W); on wasp, Mayaguez, Sept. 13, 1937, George A. Pfaffman, *7027* (W); on *Mischocyttarus cubensis*, Lares, Dec. 12, 1941, L. F. Martorell (W).

In addition to the above there are two specimens on wasps in the Mycological Collections of the U. S. Bureau of Plant Industry which were received from New Orleans, Louisiana. It is stated that the specimens were intercepted in shipments from Mexico and probably from Costa Rica. Speare (**1920**) also reports the species from California. The report of the species on *Bothriocera* sp. from Virginia by Miss Charles (**1941a**) is doubtful. The host (Homoptera) would indicate that the fungus probably is *Hirsutella citriformis*. From the records available *H. saussurei* appears to develop mostly on the social wasps. The report on an Ichneumonfly from North Carolina appears to be the only exception.

Most of the specimens of *Hirsutella saussurei* are single infected wasps. This probably indicates that generally infected individuals die away from the nest. One collection has been seen of a nest with number of infected wasps. This was brought to the writers attention by George W. Byers of the Museum of Zoology of the University of Michigan. Through him a loan of the collection was obtained from Sgt. James Gentry of the Fourth Army Area Laboratory of Ft. Sam Houston, Texas. The nest, which had been collected in Arkansas, had twelve parasitized wasps clinging to it (FIG. 7). This, however, may represent an unusual condition since the nest had been sprayed with DDT and death of the insects on the nest may have been caused by the insecticide rather than by the fungus.

Fungous parasites of wasps were noted and described as early as the first part of the 18th century. Since the descriptions in the early literature did not include microscope characters it is difficult to determine the species involved.

It seems probable that Felton (**1765**) illustrated *H. saussurei* when he described a species of wasp, *Vespa crinita,* from a parasitized specimen of wasp from Jamaica. The synnemata of the fungus are described as hairs arising from various parts of the insect. In **1817** Ditmar described *Isaria sphecophila* on a species of *Vespa* from Germany. His illustrations and description suggest a species of *Hirsutella.*

Saussure (**1858**) in connection with his discussion of diseases of social wasps illustrated and described a wasp (Pl. IX, Fig. 5) with a number of what appear to be linear synnemata arising from various parts of the thorax and abdomen. Saussure states that during the time which he spent in the tropics he found only one "quêpe vegetante" which was from Jamaica. For this he gives a description including perithecia and asci which was supplied by Lebert who placed it in a new genus and species *Polistophtora antillarum.* What connection the illustration cited by Saussure has with this species is difficult to determine. Saussure states, "l'espèce se trouva identique a celle qu'on voit figurée pl. XI, fig. 5 (*Polistes americanus*), mais j'eus le bonheur de rencontrer le cryptogame arrivé sòu étât de parfaite maturité." Lebert's (**1858**) discussion and illustrations of the species do not include a condition such as illustrated by Saussure in his Plate IX, Fig. 5 but are of a fungus bearing perithecia, evidently the specimens to which Saussure refers as in "étât de parfaite maturité." It would appear that the figure 5 of Plate IX was not drawn from a collection by Saussure and the source of the specimen on which it is based is apparently unknown. However, Cooke (**1892**) proposed the name *Isaria saussurei* pro tem. based on this illustration. Speare (**1920**) made the combination *Hirsutella saussurei* (Cooke) Speare and gave a description and illustrations based on specimens on *Polistes* sp. from the British West Indies, North Carolina, California and Hawaii. Since *Isaria saussurei* was published by Cooke as a provisional name which was based on an illustration taken from a specimen of unknown origin the validity of the name is very questionable. The only other name which might be applicable is *Isaria sphecophila* Ditm. Speare suggested that Ditmar's fungus was a *Hirsutella.* As far as has been determined a *Hirsutella* is unknown on

wasps in Europe. Petch (**1937**) states that "the available specimens in herbaria which would otherwise be referred to *Isaria sphecocephala* [error for *sphecophila*] are *Hymenostilbe*" and he published the combination *Hymenostilbe sphecophila* (Ditm.) Petch. The illustrations of Ditmar so strongly suggests a species of *Hirsutella* that the writer is reluctant to accept this conclusion. The setose condition strongly suggests the occurrence of the long sterigmata of the phialides of *Hirsutella* and the small globules between them the mucous droplets which commonly occur. However, it will be necessary to find specimens of *Hirsutella* on wasps in Europe to support this view. Under the circumstances, it does not seem desirable to propose a new name for the fungus. Therefore *Hirsutella saussurei* is employed in the sense used by Speare.

Petch (**1934**) has reported that three synnemata of *Hirsutella saussurei* were found associated with perithecia bearing clavae of *Cordyceps humberti* on a Hymenopteron from Sarawak. *C. humberti* was illustrated by Saussure, Pl. V, Fig. 9, from a wasp from tropical Africa. It has not been reported from North America. *Cordyceps sphecocephala* has been reported a number of times on wasps for North America. According to Petch its conidial stage is *Hymenostilbe sphecophila*. Only one collection of this *Hymenostilbe* from North America (Puerto Rico) has been seen by the writer (**1950**) and it is not associated with an ascogenous stage. More information is needed before the relationship of *Hirsutella saussurei* can be considered as satisfactorily established.

Hirsutella formicarum was described by Petch (**1934**) on ants from British Guiana and Ceylon. The phialides are given as ovoid bases 8–11 × 4–6 μ and sterigmata 20 μ long and the conidia as narrowly cymbiform, 9–11 × 2 μ. It occurred associated with the perithecial stage of *Cordyceps unilateralis* (Tul.) Sacc. Kobayasi (**1941**) has reported *H. formicarum* from Japan. He describes the phialides with cylindric, ellipsoid or fusoid bases 8.5–17.5 × 2.8–4.2 μ and sterigmata 7–14 μ long. The conidia are given as ovoid, ellipsoid or fusoid, 3–4.2 × 1.2 μ. It is very doubtful that this is *H. formicarum* if Petch's measurements of conidia are correct. There is some doubt concerning this since, when he described *H. formicarum*, he referred to an earlier publication (**1924**)

in which he described the conidia of the Ceylon specimens as 3–5 × 1 μ. Petch (**1937**), in a discussion of *Cordyceps formicivora* (Schroet.) Sacc. in which he concluded it was synonymous with *C. unilateralis,* has stated that a specimen (*6154*) in the Farlow Herbarium bears *Hirsutella* conidiophores accompanying perithecial clavae. This specimen has been examined. The perithecial clavae are immature. They are accompanied by two short synnemata which bear subulate phialides and ellipsoid or ovoid conidia which are 2–2.5 × 1–1.5 μ with a slight mucus and which adhere in large numbers in groups 14–60 μ in diameter. It is certainly distinct from *Hirsutella formicarum* and does not appear to be a species of *Hirsutella.* From the meager material it is not possible to definitely place it. It hardly seems possible that these are all conidial stages of *Cordyceps unilateralis.*

Hirsutella liberiana Mains (**1949**), described on an ant from Liberia, has cylindric, narrow conidia, 3–4 × 0.7–1.0 μ. It was associated with *Torrubiella liberiana* Mains.

SPECIES OF DOUBTFUL POSITION IN HIRSUTELLA

Hirsutella neo-volkiana Kobayasi (**1941**) on Coleoptera in Japan and *H. volkiana* Kobayasi (**1941**) on Coleoptera in Brazil have subulate phialides terminating hyphae and forming a dense palisade layer. They are not species of *Hirsutella* as the genus is here interpreted. *Hirsutella arachnophila* Petch on spiders from India has sterigmata only 2 μ long and no mention is made of a mucus covering the conidia according to the description given by Petch (**1923a, 1924**).

The following species have been described in *Hirsutella* without synnemata and probably should be placed in the Moniliaceae: *Hirsutella versicolor* Petch (**1932a**) and *H. strigosa* Petch (**1939**) on leafhoppers from Ceylon; *H. acridiorum* Petch (**1932c**) on a grasshopper from Great Britain; *H. aphidis* Petch (**1936**) on aphis from England; *H. nodulosa* Petch (**1926**) on *Zeuzera coffeae* from India; *H. thompsonii* F. E. Fisher (**1950**) on *Phyllocoptruta oleivora* from Florida. Speare (**1920**) in describing *H. floccosa* on *Peregrinis maidis* from Jamaica stated that it is "somewhat unlike" the other species of *Hirsutella* in that "the synnemata are merely

wart-like outgrowths arising from an external cotton-like subiculum." Petch (**1937**) has reported it on a leafhopper from North Carolina, describing phialides only on the mycelium. Fran. E. Fisher (**1950**) has described *H. besseyi* on *Lepidosaphes beckii* from Florida stating that synnemata, for which a description is not given, are infrequently produced. Specimens seen by the writer lacked synnemata. *Hirsutella verticillioides* Charles (**1937**) on *Leptopharsam heveae* from Brazil lacks synnemata and is considered by Petch (**1939**) to be the same as *Verticillium fuliginosum* Petch.

2. **TILACHLIDIUM** Preuss, Linnaea **24**: 126. 1851.

Synnemata terete, simple to much branched, cylindric, narrowing toward the apices, consisting of bundles of parallel longitudinal usually closely compacted hyphae; phialides scattered, hyaline, subulate, gradually narrowing to acuminate apices, usually terminating hyphae of the synnema, or as lateral cells of the hyphae, single or sometimes in groups of two or three; conidia hyaline, covered with a mucus, agglutinating into large spherical or irregular clumps.

Type species *Tilachlidium pinnatum* Preuss.

Tilachlidium was described by Preuss (**1851**) based on *T. pinnatum* reported on dried fungi, chiefly *Agaricus galericulatus*. He described a stilbaceous fungus having simple or branched synnemata bearing phialides which produced obovoid-oblong spores in globose, white, shining, glutinous globules. The type of this species has not been available for study. Petch (**1937**) has concluded that *Isaria brachiata* Fr. is the same fungus. Three specimens of *Isaria brachiata,* Farlow Herbarium *759,* Univ. Mich. Herb. Lowe Fl. *305,* and Syd. Mycoth. Germ. *1048,* have been available for study. It has been assumed that these are representative of the type species. The conclusions of Petch appear to be justified.

Most of the species which have been described in *Tilachlidium* are saprophytic on various substrata. Petch (**1931**) has described an entomogenous species *T. larvarum* on a lepidopterous insect. The subulate phialides and the type of clumping of the conidia of *Hirsutella ramosa* on a lepidopterous larva place that species in Tilachlidium.

Tilachlidium ramosum Mains, comb. nov. FIG. 20.
Hirsutella ramosa Mains, Mycologia **41**: 308. 1949.

Synnemata terete, composed of parallel longitudinal hyphae irregularly branched, the branches at right angles or slightly oblique often bearing secondary branches, up to 8 mm. long, 52–140 μ thick at the base slightly narrowing to the apices, the main stem light yellow below, the branches and upper part of the main stem white; phialides subulate, gradually narrowing to acuminate apices, 19.3–36.4 μ long, 2.1–3.2 μ wide at the bases, scattered on the upper portions of the main stem and branches, commonly terminating the hyphae of the synnemata, less frequently lateral along the outer hyphae of the synnemata, single or 1–3 at the apices of short hyphae; conidia oblong, 3.2–5.5 × 1–1.4 μ, covered with a slight mucus adhering to form spherical clumps of several spores.

On fragments of a lepidopterous larva on a mossy log, Salmon River, Nova Scotia, Sept. 7, 1931, L. E. Wehmeyer, *1474*, type (M).

In describing this species as a *Hirsutella* it was noted that it differed from other species in the genus in having narrow subulate phialides. The clumps of conidia which contain a number of adhering spores do not have the thick covering of mucus as in *Hirsutella* and disintegrate fairly easily.

According to the description given by Petch, *Tilachlidium larvarum* on a microlepidopteron from Ceylon has synnemata up to 1 mm. long. The phialides are simple, conical to slightly flasked-shaped, attenuated gradually upward and 14–22 × 2 μ. The conidia are narrow-oval with rounded ends, 4–6 × 2–2.5 μ, and form mucilaginous heads up to 14 μ in diameter.

3. **SYNNEMATIUM** Speare, Mycologia **12**: 74. 1920.

Synnemata simple or branched, terete, at first white, becoming brownish, consisting of parallel longitudinal hyphae; phialides subulate, terminating the hyphae, mostly fasciculate at the apices of the synnemata, some scattered laterally on the upper portions; conidia one-celled, hyaline to pale brown, light brown in mass, covered with a mucus, agglutinating into spherical droplets of several spores at the apices of the phialides; sclerotia spherical, at first white, becoming brown, composed of thick-walled cells.

Type species *Synnematium jonesii* Speare.

SYNNEMATIUM JONESII Speare, Mycologia **12**: 74. 1920. FIGS. 11, 12.

Synnemata mostly dendroid-branched, occasionally simple, terete, 100–200 μ thick, 2–3 mm. long, in agar cultures up to 20 mm. long, at first white becoming brown, especially in the lower portions of the synnemata, consisting of parallel longitudinal hyphae which are hyaline at first, becoming light brown; phialides subulate, gradually narrowing to acuminate apices, 40–50 μ long, 2.5–4 μ thick below, terminating the hyphae of the synnemata, mostly fasiculate at the apices of the synnemata, some scattered on the upper portions of synnemata; conidia oblong, somewhat narrowed but rounded at the ends, 8–10 × 2.5–4 μ, hyaline or very pale brown, produced successively from apices of phialides in a mucus, agglutinating to form spherical brownish droplets containing several spores on the scattered phialides, the droplets of terminal phialides cohering to form large spherical brownish masses 80–250 μ in diameter, containing many spores; sclerotia spherical, 90–350 μ in diameter, at first white, becoming brown, consisting of thick-walled cells.

California: on *Harpalus* sp., Auburn, Aug. 8, 1890, Atkinson (FH *694*).

District of Columbia: on *Pardomis* sp. from cedar wood, W. H. Weston, Jr. (FH *6262*).

Louisiana: on *Mezira*, Baton Rouge, T. H. Jones, type (FH *4150*); also agar cultures (FH *5263, 5369*).

Maine: on *Philonthus* sp., Kittery Point, Sept. 1893, R. Thaxter (FH *6147*).

Costa Rica: on large leafhopper, J. R. Johnston (FH *6143*).

Under number *4150* in the Farlow Herbarium there are several dried agar cultures and one specimen on an insect. In addition there are several dried agar cultures under numbers *5263* and *5369* with the statement that they are from the type culture of Speare. Since as far as has been determined other collections of Speare do not exist these should be recognized as type collections and the specimen on *Mezira* as the type. It agrees with the illustration given by Speare, Pl. 5, Fig. 5, except that the synnemata are darker in color than indicated by the photograph. According to Speare's statement, his illustration represents a young condition and later in their development the synnemata are brown. The synnemata are short, 2–3 mm., and are dendroid-branched. The synnemata have

the structure shown by Speare in his Pl. 4, Fig. 1. As is well illustrated by Speare, the phialides are subulate and terminate the hyphae of the synnema, Pl. 4, Figs. 1 and 4. A few of the hyphae end before reaching the apex of the synnema and these produce scattered phialides mostly in the upper portions of the synnema. These bear small spherical heads of several conidia in a droplet of mucus (Speare, Pl. 4, Fig. 10). The number of conidia and the irregular arrangement in the droplet resemble that in *Cephalosporium*. This differs from the condition found in *Hirsutella* where usually only one conidium is found in the mucus droplet. Less frequently two or more parallel conidia occur. Droplets containing a number of conidia similar to those usually produced in *Synnematium* occur in *Hirsutella* when droplets of adjacent phialides coalesce (Speare, Pl. 3, Fig. 16). Most of the hyphae of synnemata of *S. jonesii* on the insect do not terminate until they reach the ends of the synnemata and there they form fascicles of phialides. The droplets of spores produced by them coalesce to form large spherical aggregates which give a capitate appearance to the synnemata (Speare, Pl. 4, Fig. 1). Sclerotia were not observed on the insect.

Speare states that he was able to grow the fungus readily on potato agar. The dried cultures show certain differences from the fungus on the insect. The synnemata are longer, up to 20 mm. The hyphae of the synnemata are not as compactly bound together and more frequently terminate laterally on the synnema. As a result there are fewer terminal phialides and most synnemata do not produce the large heads of aggregated droplets. This condition is shown by Speare for synnemata arising from sclerotia, Pl. 4, Fig. 5. Many sclerotia occur in the dried cultures.

Other specimens in the Farlow Herbarium have been determined as *Synnematium jonesii* by Petch (**1937**) who has published a discussion concerning them. Numbers *6147* and *6262* have short synnemata and are without sclerotia, while numbers *694* and *6143* have sclerotia and no synnema. Petch also reports collections on *Pororeus simulans* from the Philippines, on *Basilides bipinnis* from Sierra Leone, on *Promecotheca bicolor* from Fiji and on *Helopeltis* sp. from the Belgian Congo, all having sclerotia and no synnema.

Whether these collections are all of one species is somewhat uncertain. If so, the species has a wide host range infecting insects of several orders.

Kobayasi (**1941**) has described *Synnematium graptopsaltriae*, on *Graptopsaltria nigrofuscata* (Homoptera) in Japan. In the separation of the species from *S. jonesii* Kobayasi places emphasis on the length of the synnemata, "several centimeters," the lack of sclerotia and the non-fasciculated terminal phialides of *S. graptopsaltriae*. These are all variations which occur in *S. jonesii*. His illustration indicates that the branching is different. The branches develop at right angles to the main stem instead of occurring as irregular, somewhat dichotomous divisions of the synnema.

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A DERMEA ON PRUNUS FROM CHINA [1]

J. WALTON GROVES [2]

(WITH 2 FIGURES)

In 1948, after the Mycological Society Foray held at the University of Michigan Biological Station, the writer had the opportunity of examining some of the collections of Discomycetes in the herbarium of the University of Michigan. Among these was a specimen labelled *Phaeangium pruni* Teng #3352. This fungus was collected in the Hunba Forest, Sikang, China, on *Prunus* sp. and proved to be a striking species of *Dermea*.

Three species of *Dermea* have already been recognized as occurring on *Prunus* (Groves, **1946**), but this species differs from all three both in gross appearance and in microscopic characters. The most striking character is the large size of the apothecia, which vary from 2–6 mm. in diameter and are much larger than those of any other known species of *Dermea*. The gross appearance suggests a large *D. cerasi* (Pers. ex Fr.) Fr., but the latter is usually less than 3 mm. in diameter. The other two species on *Prunus*, *D. prunastri* (Pers. ex Fr.) Fr. and *D. padi* (Alb. & Schw.) Fr., are still smaller, rarely exceeding 1 mm. in diameter.

The asci and spores of the Chinese specimen are also larger than those of the other three species. The asci and spores of *D. cerasi*, *D. prunastri* and *D. padi* are all somewhat similar in size and shape and difficult to distinguish from each other, with those of *D. padi* generally somewhat smaller than those of the other two. The asci of *D. cerasi* are the largest and may reach 150 μ in length but rarely exceed 125 μ, whereas those of the Chinese specimen are mostly 125–165 μ in length. The ascospores in the Chinese specimen are about the same length as in the other species, mostly 15–20 μ, but they are broader, mostly 8–10 μ, whereas those of the other three species rarely exceed 7.5 μ, and consequently they are a different shape.

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FIG. 1. Apothecia of *D. pruni*. M = 4 × approx.

Unfortunately, no conidial fructifications could be found in the Chinese specimen. The conidial state would, no doubt, prove to be very interesting since the conidia and conidial fruiting bodies have proved to be of great value in differentiating species in the genus *Dermea*, the other three species on *Prunus* being readily distinguished by this means. However, this Chinese specimen is so distinctive in both macroscopic and microscopic characters that it may be recognized without hesitation as a fourth species of *Dermea* occurring on *Prunus*.

Nannfeldt (**1932**) has already pointed out that the genus *Phaeangium* contains a heterogeneous assemblage of species. The type of the genus is *Phaeangium rubi* (Bauml.) Sacc. & Syd. (= *Cenangium rubi* Bauml. non Fr.). On the basis of the description, Nannfeldt suggested that this species was based on old, overmature specimens of *Pezicula rubi* (Lib.) Niessl, and that consequently *Phaeangium* was a synonym of *Pezicula*. However, it seems to me that the description agrees much better with *Velutaria rufoolivacea*

(Alb. & Schw.) Fckl., also occurring on *Rubus*, than it does with *Pezicula rubi*. If this conclusion is correct, *Phaeangium* would become a synonym of *Velutaria* rather than of *Pezicula*. In any event, it seems unlikely that the genus *Phaeangium* can be maintained, but its exact status cannot be determined until the type has been re-studied. *Phaeangium pruni* Teng is unquestionably a good *Dermea* species.

FIG. 2. Drawing of asci and ascospores.

Dermea pruni (Teng) comb. nov.

Phaeangium pruni Teng, Sinensia **11**: 109. 1940.

Apothecia erumpent, scattered to gregarious, separate or sometimes two or three in a cluster, circular to undulate, 3–7 mm. in diameter, 1–2 mm. in height, excipulum dark reddish-brown to black, slightly furfuraceous, hard, leathery to horny in consistency, becoming more fleshy-leathery when moist; hymenium black, at first concave, becoming plane to strongly convex, slightly rough, at first with a thick, raised, brownish margin which later disappears, more fleshy than the excipulum; tissue of the hypothecium prosenchymatous composed of interwoven, ascending hyphae about 5–12 μ in diameter with the wall thickened and slightly gelatinized,

curving toward the outside to form a thick, but not sharply differentiated, pseudoparenchymatous excipulum composed of almost iso-diametric, thick-walled, hyaline to yellowish cells, mostly about 5–10 μ in diameter, and arranged in oblique to almost horizontal rows; sub-hymenium a fairly thick, compact zone of closely interwoven, hyaline hyphae about 2–3 μ in diameter; asci cylindric to cylindric-clavate, fairly long stalked, eight spored, (115–)125–165 (–175) × (12–)14–16 μ; ascospores ellipsoid-fusiform to ovoid, one- (to four-) celled, straight or slightly inequilateral, hyaline, soon yellowish-brown, uniseriate or partly biseriate, 15–20 × (7–) 8–10 μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.5 μ in diameter, the tips swollen up to 4.0 μ and glued together forming a brownish epithecium.

Host: *Prunus* sp.

The single specimen seen is in the Herbarium of the University of Michigan. It is labelled Reliquiae Herbarii Fungorum Sinensis, Hunba Forest, Kiulunghsien, Sikang. Coll. & det. S. C. Teng *3352*. 5–12–39. In the original description by Teng (**1940**), the number cited was *3352*, hence this would appear to be part of the type. A note attached to the specimen states "undestroyed portion of the Chinese National Fungus Herbarium given to the Bureau of Plant Industry and Cornell University, 1939–40."

The author is indebted to Mr. Wan-nien Siang, Pullman, Washington, for his kind assistance in obtaining the original description.

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AN EXAMPLE OF SYNERGISTIC GROWTH INHIBITION BETWEEN ROOT-INHABITING FUNGI

DOUGLAS R. REDMOND AND VICTOR M. CUTTER, JR.

(WITH 1 FIGURE)

During a study of the interrelationships of root-inhabiting fungi and the occurrence of "dieback" of yellow birch (*Betula lutea* Michx.) in Nova Scotia, *Mortierella alpina* Peyronel and two unidentified imperfect species, which have never been observed to sporulate, were the most commonly isolated fungi. When these three fungi were grown singly and in all possible combinations in mixed shake culture they produced a very unusual pattern of growth inhibition which seemed worthy of further investigation.

The three organisms used were all isolated in 1950 from dying roots of yellow birch at Lake O'Law, Inverness Co., N. S. The fungi were grown singly and in all possible combinations in 20 ml. of liquid media in 125 ml. Erlenmeyer flasks shaken continuously on a reciprocating shaker at 104 strokes per minute at 23° C. The medium used contained 20 gm. dextrose, 5 grams Difco yeast extract and 1000 cc. distilled water. The medium was decanted from the flasks after 14 days and cellulose penicillin assay disks were dipped into the culture media and placed on the surface of agar pour plates of the same medium plus 1% agar heavily inoculated with a suspension of ground mycelium and spores of *Mortierella alpina*.

The media from A and B grown separately or together inhibited growth of *M. alpina*. To test the thermostability of the active principle an aliquot of the medium from each of these cultures was autoclaved at 15 lbs. pressure for 15 minutes at 125° C. In addition equal amounts of culture media from A were mixed with media from B, both before and after autoclaving. These media and mixtures of media were all tested in quadruplicate for their possible ability to inhibit growth of *M. alpina*. Since *M. alpina* showed no inhibition against A or B, it was not tested further.

Table I indicates that media from both A and B grown separately inhibit the growth of *M. alpina*. However, this inhibitory principle is not stable at 125° C. When A and B are cultured together a heat-stable product is synthesized which strongly inhibits the growth of *M. alpina*. The culture media from A and B grown separately and then mixed either prior to or after autoclaving do not exhibit a similar inhibitory activity. However, when media from A and B are mixed before autoclaving a very transient inhibition of *M. alpina* does occur which suggests that heating of the combined media results in the liberation of another toxic component.

TABLE I

RADIUS OF INHIBITION ZONE IN MMS. ON POUR PLATES OF *M. alpina*

	Test No.	A	B	AB grown together	A added to B	
Unautoclaved media	1	4.5	2.5	10.0	4.5	
	2	4.5	6.5	10.5	5.5	
	3	8.0	9.0	12.0	6.0	
	4	5.5	6.5	10.5	6.5	
Mean		5.63	6.00	10.75	5.63	
					after autoclaving	before autoclaving
Autoclaved media	1	0.0	0.0	11.0	0.0	*5.0
	2	0.0	0.0	12.0	0.0	*1.5
	3	0.0	*2.0	12.5	0.0	*2.0
	4	0.0	*2.0	12.5	0.0	*2.0
Mean		0.0	*1.0	12.00	0.0	*2.63

* Transient inhibition.

Passing culture media from A and B grown individually through Seitz cellulose filter pads substantially reduced the inhibitory effect. This indicates that the heat-labile inhibitory substance is readily adsorbed on cellulose. However, the inhibitory effect of the thermostable component was not significantly reduced by passage through a Seitz filter pad.

In another experiment the three cultures were grown in bags made of cellulose sausage casing [1] suspended in the shaken medium as indicated in FIG. 1, A. This method is applicable for separating

[1] Obtained from Visking Corp., 6733 W. 65th Street, Chicago, Ill.

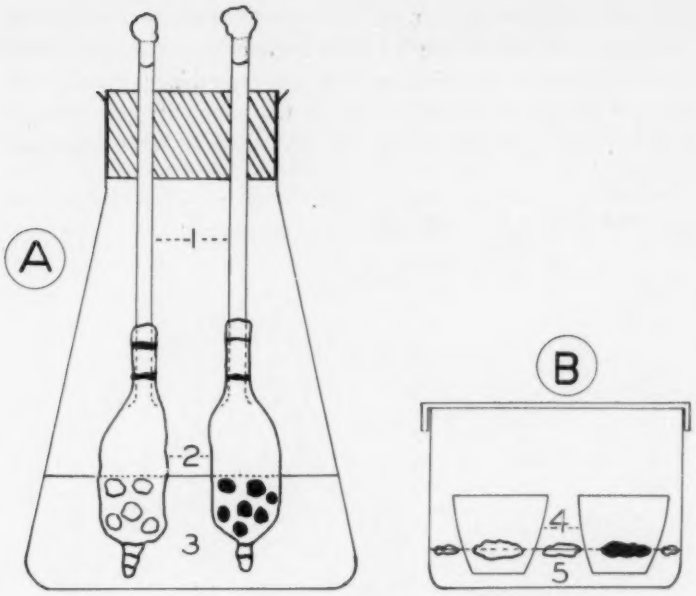

FIG. 1. A. 1. Glass tubes. 2. Cellulose bags. 3. Medium.
B. 4. Alundum crucible. 5. Agar medium.

any organisms which are not capable of degrading cellulose. The fungi were also segregated in a common vessel by the use of Alundum crucibles embedded in an agar substratum as shown in FIG. 1, B. It is important to use crucibles of sufficiently fine porosity to prevent the passage of fungus hyphae through the pores. It is necessary to segregate the organisms to permit accurate individual growth measurements.

The results of these experiments, which confirm those presented in TABLE I, indicate that the inhibitory synergistic effect upon *M. alpina* occurs when A and B are grown in a common medium regardless of whether their respective mycelia are intermingled.

The occurrence of synergistic interrelations between microorganisms is perhaps more common than is ordinarily suspected and may be of considerable importance in determining the distribution and survival of certain species in particular habitats. The technique described above offers a simple method for the rapid detection of

such interrelationships. In the example cited the mutual production of an antibiotic principle by two organisms, which individually cannot synthesize the substance, suggests that this approach might be more widely exploited because of the possibility that valuable new antibiotic substances may be produced in such synergistic complexes.

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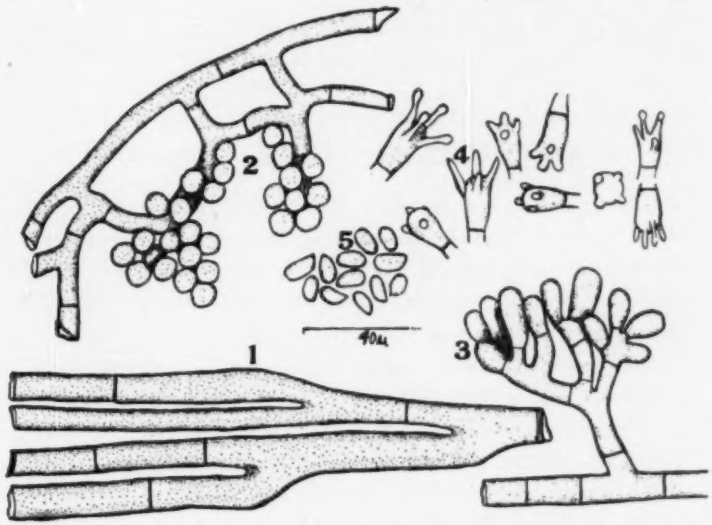
BRIEF ARTICLES

CORTICIUM MICROSCLEROTIA NOM. NOV.

In 1939 the writer published [1] a description of *Corticium microsclerotia* (Matz) n. comb., the basidiomycetous stage of *Rhizoctonia microsclerotia* n. sp., described by Matz [2] and listed by Saccardo [3] without an accompanying validating latin diagnosis, to which



FIGS. 1–5. *Corticium microsclerotia*, the thread-blight fungus. 1. Strands of superficial hyphae. 2. Basidiomycetous mycelium and top view of young basidia. 3. Young basidia. 4. Stages in development of basidia. 5. Basidiospores.

Rogers [4] referred as a *nomen nudum*. In recent conversations, Rogers, Martin and the writer have discussed the desirability of supplying the omitted latin translation, which was prepared by Rogers.

[1] Weber, G. F. Web-blight, a disease of beans caused by *Corticium microsclerotia*. Phytopath. **29**: 559–575. 1939.

[2] Matz, J. A rhizoctonia of the fig. Phytopath. **7**: 110–117. 1917.

[3] Saccardo, P. A. Sylloge Fungorum, Supplementum universale. **25**: 1003. 1931.

[4] Rogers, D. P. The genus *Pellicularia* (Thelephoraceae). Farlowia **1**: 95–118. 1943.

This offering is made in conformity, according to Stevenson & Rogers,[5] with the new text supplied for Article 57 in the International Rules for Botanical Nomenclature.

Sclerotia hyphis in hospitis superficie adnatis insidentia, 80–300 × 80–600 μ, plerumque 200 × 350 μ, subglobosa vel elongata; hyphae subepidermales 4–6 μ diam., hyalinae, septatae. Basidiosporae ovoideae, hyalinae, tenuiter tunicatae, basi apiculatae, 5–6 × 9–11 μ.

Hab. in plantis lignosis perennis atque fabis annuisque variis. Gainesville, respublicae Floridae.

Type specimens No. 7850 in Florida Agricultural Experiment Station Herbarium.—GEORGE F. WEBER.

A NOTE ON DICTYUCHUS PSEUDODICTYON [1]

In 1926, Coker and Braxton (Jour. Elisha Mitchell Soc. **42**: 144–146) isolated and described a dictyoid fungus from soil near Chapel Hill, N. C., but did not apply a name to it. In 1931, in a publication by Couch (Jour. Elisha Mitchell Soc. **46**: 228–229), Coker named this fungus *Dictyuchus pseudodictyon*. Since that time the fungus has been isolated twice from soil collections made in 1949 in Leon County, Florida (Ziegler, Contr. Sci., Fla. State Univ. Studies, in press), and from soil from a swamp near Sebring, Florida by Johnson (Mycologia **43**: 365–372. 1951).

Both investigators realized that the characteristics of their respective isolates differed considerably from those given in the original description of *D. pseudodictyon*. It was at once obvious that there was little similarity between illustrations of the characteristic features of Coker and Braxton's fungus and those of the Florida isolates. Both authors considered publishing their respective forms as new species, which, had it been done, would have confused the situation considerably. Johnson, however, merely described his fungus as an unidentified isolate (designating it as *Dictyuchus* 521) suggestive of *D. pseudodictyon*, while Miss Linthicum and Ziegler studied some physiological aspects of their iso-

[5] Stevenson, J. A., and D. P. Rogers. Mycological nomenclature at the 7th International Botanical Congress. United States Dept. Agr., Plant Disease Reporter **34**: 327–333. 1950.

[1] Contribution No. 47, Botanical Laboratory, Florida State University.

lates (Jour. Elisha Mitchell Soc. **68**, in press). On comparison, the authors have found that the two strains from Leon County and *Dictyuchus* 521 are the same fungus, which requires a somewhat broadened interpretation of the original description of *D. pseudodictyon.*

The writers propose, therefore, to adopt the description of *Dictyuchus* 521 as that of the species, *Dictyuchus pseudodictyon.* This, in effect, emends the original description of the latter to include the Florida strains. We believe that the proposed course will not only eliminate the confusion which undoubtedly exists as to the true identity of the fungi in question, but will also indicate something of the variability to be expected in other isolates of *D. pseudodictyon.*—T. W. JOHNSON, JR., A. W. ZIEGLER AND BETTY LINTHICUM.

REVIEWS

MUSHROOMS OF EASTERN CANADA AND THE UNITED STATES (HOW TO RECOGNIZE AND PREPARE THE EDIBLE VARIETIES), by René Pomerleau in cooperation with H. A. C. Jackson. 302 pp., 5 roto-calcographic plates, 7 pages of line-drawings, 58 pages of photographs. Les Éditions Chantecler Ltée., Montreal. 1951. Price, $7.00.

This is a popular treatise and handbook which will be very useful because of its scope and general excellence. It was prepared in French to stimulate the use of wild mushrooms by French-Canadians and then fortunately translated into English and published in both languages to give it wider use in the northeastern portion of this continent. The author is Dr. René Pomerleau, Director of the Division of Forest Pathology in the Province of Quebec, Fellow of the Royal Society of Canada, Professor of Forest Pathology and Mycology at Laval University and Lecturer at the Botanical Garden of Montreal, who is a competent student of the fungi in general as well as a well-known forest pathologist. He has written in a plain and easy style that will appeal to the general public. He was fortunate in having available for the English edition the services of Dr. Naomi Jackson, daughter of the illustrator, whose literary gifts and training transformed the original manuscript into something completely and fluently English instead of a work-a-day translation of the sort so often met with.

After a chapter of general information on fleshy fungi, their characters and structure, brief descriptions with keys are given for 126 agarics, 18 boleti, and 26 other edible or non-edible forms or groups. At the end of the book are chapters on gathering, preparation for the herbarium, selection and preparation for the table, and mushroom poisoning. There is a brief glossary, and finally complete lists and indices of everything in the book.

The work is well illustrated. In addition to 7 plates of line drawings for purposes of edification, there are 122 reproductions

of photographs, almost all clear and good for satisfactory aid to the amateur. In addition, there are 5 colored plates of 44 species, 31 of which are agarics. The original colored drawings were made by that superb artist and naturalist, Henry A. C. Jackson, of Montreal, brother of A. Y. Jackson, the famous Canadian artist, and a very close friend and collaborator of mine. In my opinion, there have never been colored drawings of fungi either or both so artistically beautiful and so accurate mycologically as those done by Harry Jackson. It may be of interest to note that Pomerleau's volume was reviewed by the art critic of the Montreal Star, who remarked that although the book was not an art book he was amply justified in including this work on mushrooms in his column. The reproductions of the colored drawings are satisfactory and certainly will be valuable as well as very pleasing to users of the book.

The format is excellent, with an especially pleasing type. The book is handy, though not of the pocket type, and from every point of view is appealing and ought to find wide use.—WALTER H. SNELL.

CHAMPIGNONS COMESTIBLES (FUNGI EDULES), by Guillermo Herter. 203 pp., 101 pl. Paul Lechevalier, Paris. 1951. Price, 1800 fcs.

The author, known for his studies in South American botany but resident in Switzerland in recent years, has prepared this volume as a guide to the more important edible fungi of central Europe. Excellent drawings and concise but clear descriptions of 101 edible species, most of which also occur in the United States and Canada, are given. The drawings show various stages of the fructifications, reproduced at half natural size, and also the spores, × 1000. No poisonous species are illustrated but reference to them is made in connection with edible species for which they may be mistaken. For certain edible forms, notably the Helvellaceae, caution is urged. Full lists of French and German common names are given.

REPORT OF THE MANAGING EDITOR OF MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1951

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$2,308.00	
Other subscriptions	3,897.84	
Sales of vol. 25 and later	777.07	
Excess pages	290.18	
	$7,273.09	
Special funds		
Sales of vol. 1–24	$ 95.00	
Income from endowment	690.00	
	$ 785.00	
Total receipts		$ 8,058.09
Cash disbursements		
Printing and distribution	$5,346.34	
Office expense	338.79	
Total expenditures		$ 5,685.13
Excess of receipts over expenditures		2,372.96
Unexpended balance July 1, 1950		4,649.48
Unexpended balance June 30, 1951		$ 7,022.44
Endowment		$14,000.00

The above MYCOLOGIA funds are administered by The New York Botanical Garden. The balances at June 30, 1951, are (except for printing and distribution expense of $1,318.21 for the May–June, 1951, issue, which had not been paid at June 30, 1951, although accrued in the books of The New York Botanical Garden) in agreement with the amounts shown in the financial statements of that organization which have been examined and reported on by Price Waterhouse & Co.

D. P. ROGERS, *Managing Editor*

CORRECTIONS TO VOL. XLIII

No. 1, p. 4. In the list of Dr. Zundel's papers, the first reference to no. 32 should be deleted and should be replaced by:

31. Additions and corrections to Ustilaginales. North Amer. Flora **7**: 971–1045. 1939.

No. 3, p. 284. Footnote 1 should read: L. Georg. Personal communication. 1949.

P. 286, l. 7. For the phrase "is distilled water" read "*in* distilled water."

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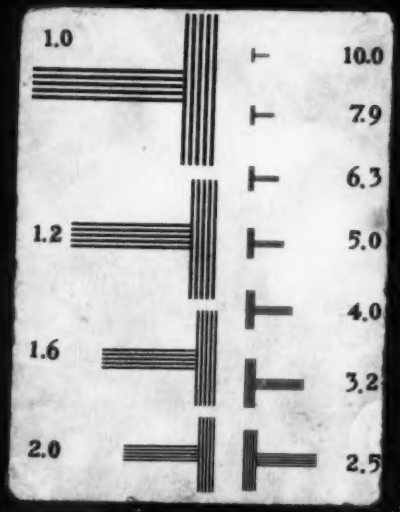

100 MILLIMETERS

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